PD-ABP-330

EVALUATION REPORT

Basic and Applied Research

in

Tropical Diseases

of the

Middle East

1993-1996

Middle East Regional Cooperation (MERC) Project

PD-ABP-330

Report

of the

External Evaluation

of the

USAID-NIAID

Middle East Regional Cooperation (MERC) Program,

Basic and Applied Research in Tropical Diseases of the Middle East

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PROJECT DATA SHEET

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EXECUTIVE SUMMARY

BACKGROUND: In October 1993 the National Institute of Allergy and Infectious Diseases (NIAID) officially began a new phase of the Middle East Regional Cooperation Project. The PASA between NIAID and AID/W is entitled "Basic and Applied Research in Tropical Diseases of the Middle East". This project creates a network of eight individual sub-projects in five Middle Eastern countries (Morocco, Tunisia, Israel, Jordan and Lebanon). In addition to the individual projects there are collaborative (joint) activities which include common work plans for research conducted in unison, joint workshops to standardize the protocols and procedures throughout the network, consultations and technical assistance between and among network members and semiannual meetings of the Principal Investigators.

As its first activity NIAID developed and released a Request for Proposals (RFP) for this project. Forty-four copies of the RFP were sent out, and twenty-nine proposals were received and evaluated for scientific merit and feasibility of cooperation within the network. Although eighteen of them scored well enough to be funded, there were sufficient funds to make only eight awards.

The eight projects are divided into two areas of research: leishmaniasis and echinococcosis. Each research area has a scientific coordinator, who works together with NIAID staff to manage and administer the project. The Leishmaniasis Coordinator is Dr. Peter Melby of the University of Texas Health Science Center in San Antonio. The Echinococcosis coordinator is Dr. Peter Schantz of the Centers for Disease Control. This organization represents a multilateral rather approach. Rather than pairing Arab and Israeli scientists in strictly bilateral partnerships, the network activities are undertaken by the group as a whole.

Within each research area the PIs meet with their coordinator every six months, usually in conjunction with an international conference, to develop a common work plan in the context of their individual projects. Elements of the common work plans have included the conduct of workshops, joint efforts (multi country comparison of the coproantigen assay for diagnosis of canine echinococcosis) and development of a background review of leishmaniasis in the Mediterranean basin. Reciprocal training and collaboration visits have been made among all projects in the network and consultants have been brought in to address common issues.

REVIEW:

1. NIAID Administration: NIAID has been intimately involved in promoting the successful operation of this NIAID MERC project.

- The NIAID has fostered excellent coordination and collaboration among the sub-grantees in this project, largely due to the emphasis that has been placed on the establishment and adherence by all participants to the common work plan. The NIAID also has been successful in promoting frequent joint meetings among the PIs, both in the Middle East and elsewhere. The committee recommends that NIAID be vigilant for opportunities to obtain technical assistance and/or training from other MERC participants when reviewing future sub- project work plans.

- The NIAID mechanism for disbursing funds for this project -disbursements are contingent on submission of evidence of successful completion of research goals - has worked very well, and should be adopted as a model for other USAID MERC

projects. The committee suggests that NIAID modify their policies to make disbursement of funding contingent upon evidence of progress made toward the *original* goals of the program and avoid too much shifting of the projects' effort.

- The evaluation committee recognizes the desirability of incorporating a US institution such as the NIAID into MERC projects to provide just this kind of support. However, an equally important role of the US institution is eventually to wean the Middle East institutions from dependence on them, as a means of insuring that the regional collaboration network established during the project continues to function after the grant period has ended. The committee feels that NIAID should now attempt to promote direct communication between the sub-grantees whenever possible.

2. Cooperation: The review committee was impressed by the extent to which the NIAID MERC program has succeeded in fostering collaborative relationships among the Arab and Israeli members of the network. Several factors were instrumental to this success. First, the review committee felt strongly that the multilateral organization was the most important factor in the subsequent success of the program. The multilateral approach has also allowed the scientists involved to interact to a much greater extent, and with much greater success than would have been possible under a structure involving bilateral relationships alone. Several other factors have been important in supporting the cooperation in the NIAID MERC program. These include the biannual PI meetings, the workshops sponsored by the NIAID for the standardization of reagents and methods, and the short term consultancies carried out to introduce the participants to new techniques to support the efforts of the program.

Technical Progress:

SubProject #1: Institution: Faculty of Medicine, Hassan II University, Casablanca
Principal Investigator: Nouzha Guessous, M.D.

Objectives: The specific objectives are: 1) to identify regions of risk of visceral, canine and cutaneous leishmaniasis in the North of the country; 2) to study the epidemiological mechanisms of leishmaniasis transmission (vectors, man, reservoirs) in the identified foci; and 3) to develop animal models for maintaining *Leishmania* strains isolated from the field, and initiate immunological studies.

Critique: Of the sub-projects reviewed, the Moroccan project was the most comprehensive and most impressive. Under the direction of Prof. Guessous Idrissi, two new laboratories, one in Casablanca and one in Rabat have been established and two complementary and multidisciplinary teams have been trained. The Moroccan teams have essentially accomplished their first objective and significant portions of the second and third objectives. It is reasonable to expect they will accomplish the remaining portions of objectives two and three by the end of the project's third year. Collectively, the Moroccan teams have given 26 oral or poster presentations at national, regional and international scientific meeting, co-authored one technical handbook with Israeli partners, published one manuscript (Revue Marocaine de Medecine et Sante), submitted five manuscripts (Am. J. Trop. Med. Hyg, Trans. R. Soc. Trop. Med. Hyg., Parasite), completed two veterinary masters theses and initiated 5 Doctor of Science theses. The Moroccan team probably has done more to foster cooperation with other MERC Program participants and to support the common work plan than any other participant.

Sub-Project #2: Institution: Hebrew University, Jerusalem
Principal Investigator: Charles Jaffe, Ph.D.

Objectives: 1) to develop tools for rapid and precise diagnosis of leishmaniasis for epidemiological surveys, 2) to conduct epidemiological surveys of human and canine populations in northern Israel for leishmaniasis, and 3) to study the immunology of leishmaniasis in mouse models.

Critique: Dr. Jaffe has been one of the most successful MERC program PIs in establishing collaborative arrangements with other members of the MERC program. The interaction between the Israeli investigators and the local population of the Tamra area, which has developed as a consequence of the project, was very positive. The fact that the project involves diverse ethnic and cultural groups - people from Israeli, Arab and Druse settlements - is unique. Our site visit showed that the cooperation between the local health care system and the Israeli investigators was very good. Hopefully, this spirit of cooperation can be extended to dealing with other public health problems. There are a number of discrepancies between the objectives of this research project as originally outlined in the proposal, and the results as reported in the technical reports. In spite of the inconsistencies between stated research objectives and the work reported, the project has been productive

Sub-Project #3: Institution: Yarmouk University, Irbid, Jordan
Principal Investigator: Shaden Kamhawi, Ph.D.

Objectives: to perform a comprehensive study of the epidemiology of cutaneous leishmaniasis in a sporadic focus situated in North Jordan by determining the true prevalence and annual incidence of cutaneous leishmaniasis in the North Jordan focus; 2) isolating and characterizing the *Leishmania* species from human cases in this focus; and 3) identifying possible vectors of cutaneous leishmaniasis in the focus and attempt to incriminate one or more of the suspected vectors.

Critique: During the first two years of this project, the research team has already met their first two objectives (documenting the incidence of Leishmania in this area and characterizing the species involved). They also have made significant progress towards achieving their third objective (identifying possible vectors and incriminating the principal vector for *Leishmania tropica*). It is commendable that the project intends to not only continue its initially proposed activities, but also will expand its objectives during the final year. During the sand fly season, efforts will be intensified to obtain infected females and any cultures obtained will be probed using PCR techniques. Furthermore, an intensive mammal trapping effort will be conducted following the sand fly season in an effort to identify the reservoir species in this area. The committee feels that the investigators have conducted exemplary field work and solid laboratory analyses in the execution of this project to date. They seem to have a clear grasp of the important questions that remain and show every indication that they will address these questions in the coming field season. The project has excelled in providing training for students, both from Jordan and throughout the Middle East. The publication and presentations arising from this project seem adequate considering the time the project has been underway. The results obtained to date and those anticipated should have substantial relevance to the control of cutaneous leishmaniasis caused by *Leishmania tropica* in North Jordan and other regions of the Middle East where this species is important.

The project has contributed to the design and implementation of the common MERC work plan. Researchers involved with the project have participated adequately in joint meetings sponsored by the MERC program. The sponsorship of the next Principal Investigator's meeting by the Jordanian Principal Investigator also is commendable. The committee feels that the geographic position of the Jordanian project, as well as the

expertise of the research team there, gives this project the potential to play a pivotal role in the facilitation of collaboration, especially with the Lebanese and Israeli MERC projects.

Project #4 Institution: Hebrew University, Jerusalem
Principal Investigator: Yosef Schlein, Ph.D.

Objectives: 1) to examine the role of parasite chitinases in development of infection in the sand fly gut and subsequent transmission by sand fly bite; 2) to assess the effects of plant feeding on the potential for *Leishmania* transmission by sand flies, identify the actual plant food sources of *Phlebotomus papatasi* in the Jordan Valley leishmaniasis focus, and assess the importance of the local flora on the rate of leishmaniasis transmission in the endemic region; and 3) to identify the reservoir animals and sand fly vectors of *L. tropica* in the mountainous region near Jerusalem to the end that specific control measures against the disease may be employed.

Critique: The Israeli team, under the direction of Dr. Schlein, has accomplished major portions of all three objectives through rather innovative, original and productive research, and it appears likely they will accomplish the remaining portions of all three by the end of the third year. In the process they have developed new skills and techniques that will not only benefit their future studies but those of other MERC participants. Three publications in international scientific journals and two oral and one poster presentation at an international scientific conference have resulted from this research so far. Due to the nature of the basic research conducted thus far, this project has contributed marginally to the design and implementation of the common MERC plan. Other than participation in semi-annual PI meetings, formal cooperation with Arab counterparts has been rather limited. [Editor's Note: In the period immediately following this evaluation the PI traveled to Morocco to meet with other scientists from the network in an entomology consultation.]

Sub-Project #5 Institution: Institut Pasteur de Tunis
Principal Investigator: Koussay Dellagi, M.D., Ph.D.

Objectives: The original objectives were 1) comparative evaluation of classical and modern tools for diagnosis and eco-epidemiological investigation of leishmaniasis. 2) evaluation of risk factors for development of visceral and cutaneous leishmaniasis; and 3) identification of vectors and reservoir hosts involved in transmission of leishmaniasis in Tunisia.

Critique: A great deal of work was done in attempting to further purify some of the antigens, especially P32. In addition, sera were tested with different combinations or mixtures of antigens in efforts to improve sensitivity and specificity of tests. However, the rationale of some of these experiments, and their relevance to the overall goal of the project were not clear. In particular, given the fact that none of the purified antigens appeared to result in an assay that significantly improved on the crude antigen ELISA leads one to question why such a large amount of effort was directed towards following up this avenue of research. A more practical strategy of simply selecting a crude antigen and defining the best conditions for its use, even if it has some disadvantages, would have perhaps served the overall objectives of the project better. Although it does not easily fit into the category of a risk factor for development of disease, the study done by Dr. Hechmi Louzir on cytokine expression of inflammatory cells into lesions of cutaneous leishmaniasis was well-planned and carried out. Identification of vectors and reservoir

hosts involved in transmission of leishmaniasis in Tunisia. Of the three major objectives in the original proposal, this was the least developed at the time of the site visit.

Drs. Dellagi and Guizani attended the Principal Investigators' meetings in Bethesda. DNA preparations from Lebanon were sent to the Tunis lab and checked as to species by Dr. Guizani. Similarly, serum samples from Dr. El-On of Israel and 18 serum samples from Dr. Salti of Lebanon were sent to the Pasteur Institute of Tunis for testing as part of the project. The Tunisian laboratory has hosted visitors from Jordan, Morocco and Lebanon for consultations about techniques used in the MERC protocols. An epidemiology workshop for MERC program members is scheduled to be held in Tunis in May, 1996.

Sub-Project #6: Institution: Ben Gurion University of the Negev
Principal Investigator: Joseph El-On, Ph.D.

Objectives: 1) To determine the prevalence of *E. granulosus* infection in humans and dogs in another study site geographically separated from the previously identified focus of Yirka, 2) to evaluate and refine the current diagnostic methods for *E. granulosus* infection and 3) to assess parasite specific cellular and humoral immune responses to *E. granulosus*, and to follow any changes in these responses as a result of surgical or drug treatment.

Critique: The investigators of this project have made substantial progress towards answering the questions posed in the original proposal. They have demonstrated that *E. granulosus* represents an emerging health problem in Northern Israel, and have mechanisms in place to measure the effect of various control measures on the prevalence and incidence of infection. However, the laboratory studies, and in particular those involving the cellular immune responses induced by *E. granulosus* infection, have proven less fruitful than the epidemiological studies. It is suggested that these studies be re-evaluated and redirected in light of the results obtained to date. No publications have resulted from this project at the current time. However, the authors have presented their results at international meetings, and plan to publish their data on the coproantigen study in the near future. Thus, although the productivity as measured by publications is not great, this should be re-evaluated at the end on the project period.

Dr. El-On and his group have participated in the multicenter evaluation of the coproantigen assay carried out in conjunction with scientists in Jordan and Tunisia. They have also collaborated with the Tunisian group in identifying a common work plan for carrying out the epidemiological studies of hydatid disease to be carried out by the MERC program. Dr. El-On has attended all of the MERC joint meetings held to date.

Sub-Project #7: Institution: Institut Pasteur de Tunis
Principal Investigator: Riadh Ben Ismail, M.D., Ph.D.

Objectives: The overall goal of this project is to perform a detailed study of the ecology and epidemiology of cystic hydatid disease. The information gained from this study will be used to design a targeted control program. To reach this overall goal the specific aims of the project are 1) to study the prevalence and distribution of *E. granulosus* infection in humans and dogs, which serve as the definitive host of the infection, 2) to estimate, through prospective studies, the stability of the disease foci in both humans and

dogs, and 3) to identify the major routes for dispersal of the eggs of the parasite, and to investigate various human food sources for contamination by parasite eggs.

Critique: The investigators have spent the past two years attempting to collect topological and census data, and in attempting to refine the diagnostic methods needed to carry out the major specific aims of the project. Thus, the major goals of the project remain to be addressed. It is hoped that in the final year of the project the investigators will make it a priority to apply the knowledge obtained during the first two years to address the major aims of the project. The review committee recognized that the assays available to the PI are imperfect in the sense that they may lack the versatility to reliably diagnose individual cases under different rates of infection prevalence. However no data have been presented to suggest a dramatically better assay will soon be available. The committee feels that the current assays are sufficiently reliable to allow the PI to begin to collect prevalence data that will allow him to begin to test the major hypotheses of the proposal. This is that the prevalence of hydatid disease is highly focal, and most cases will be concentrated in areas where improper slaughtering practices are maintained. The committee thus recommends that Dr. Ben Ismail utilize the data and tools he currently has on hand to begin to address the major goals of the original proposal.

This project has adhered to the common work plan put forth by the MERC committee. The Tunisian group has provided antigens for testing of the HCF ELISA assay to the Israeli group, and participated in the joint evaluation of the coproantigen assay in conjunction with the Israeli and Jordanian groups. The group will also sponsor the MERC GIS workshop involving all of the MERC groups later this year. The PI has also participated in the joint meetings held by the MERC program during the past two years.

Sub-Project #8: Institution: American University of Beirut
Principal Investigator: Nuha Salti, M.D.

Objectives: The overall goal is to conduct a detailed assessment of the epidemiology and ecology of both cutaneous and visceral leishmaniasis in Lebanon. Information derived from the study will be used to design control strategies to improve the health of the Lebanese people. Specific objectives toward reaching this goal are 1)) to conduct a national human epidemiology survey, 2) to identify potential vector sand flies associated spatially and temporally with leishmaniasis cases, and 3) to identify potential reservoirs associated spatially and temporally with leishmaniasis cases.

Critique The better part of the first two years effort was devoted to achieving the first objective, i.e, preparing for and conducting a national human epidemiology survey. The level of awareness of the disease throughout the country is relatively low but is improving as a result of contacts made with local officials and health care professionals, and as a result of the "propaganda" campaigns carried out in villages and communities where suspected cases were reported.
Of concern is the possibility of outbreaks of leishmaniasis due to *L. tropica* that might be introduced by the ½ million migrant workers from Syria. The project should focus some of its attention to determining if conditions are in place to support potential epidemics of *L. tropica* in the future. The team should focus on developing more collaborations within the MERC network to confirm parasite and sand fly identification. A well organized effort has been implemented to achieve the second objective, namely, to identify the potential vectors of leishmaniasis in Lebanon. A poster presentation on the sand flies associated with leishmaniasis case sites in Lebanon was presented at the 1995 annual meeting of the American Society of Tropical Medicine and Hygiene, San Antonio, Texas, and a manuscript is being prepared for publication. Efforts to incriminate reservoir animals

have been limited to a consultation visit by Dr. Richard Ashford. During the final year of the study, special emphasis should be given to dogs as potential reservoirs of *L. infantum.* At the same time, the team should try to collect and examine a wide variety of animals associated with sand fly habitats in infected villages.

The southern portion of the country is still occupied by Israel and the Hesbollah faction is still powerful, making direct collaboration with Israeli counterparts very problematic. Of the MERC projects, the Lebanese project shows the lowest level of collaboration with the Israelis. The team's reliance on expertise of consultants from outside the MERC network has not contributed to development of ties within the network. However, the PI has exchanged samples and data with, and received monoclonal antibodies from, the Israelis indirectly through NIAID. DNA extracted from parasite cultures has been sent to Dr. Ikram Guizani, Institut Pasteur, Tunis for assistance with typing. Lebanese investigators have participated in semi-annual PI meetings and the PI participated in the workshop in Casablanca that was jointly sponsored by the Moroccans and the Israelis. Expertise is available within the MERC network for confirming parasite identification, vector identification, etc. and the Lebanese team needs to strive to open avenues to tap that expertise.

INTRODUCTION

A CONTEXT OF THIS PROJECT:

The goals of the Middle East Regional Cooperation (MERC) Program are to promote peace between Israel and its Arab neighbors and to facilitate development that will improve the well being of the people of the Middle East.

The premise of the MERC program is that people who work together in a truly collaborative manner to solve common problems or to develop shared opportunities substantially enhance their knowledge and understanding of each other, of their respective cultures and heritages, and their common goals and aspirations. A further premise of the MERC program is that participating country sectoral development programs are strengthened and enhanced by regional cooperation projects in collaborative technological or scientific efforts focused on common economic or social development priorities. A corollary is that if successful, regional cooperation projects can help attract additional financial resources to common development programs from both private and public sources.

Projects supported by the MERC program and the results produced by these projects and by those participating in them are likely to become important examples and focal points for the development and spread of further mutual understanding to colleagues within participating institutions and nations, and to other entities within the region. Active, focused, and broadly based regional cooperation among the countries of the Middle East is, therefore, the fundamental goal of the MERC program. Thus defined, regional cooperation is the principal goal of MERC projects and cooperation is an integral feature of all stages of project activities, from conceptualization and planning through implementation to completion.

B. ACTIVITIES TO BE EVALUATED

1. Background:

After consultation with scientists from the Middle East the National Institute of Allergy and Infectious Diseases (NIAID), identified five diseases that represent the most pressing health problems facing their countries. NIAID issued a request for proposals from scientists in the Middle East to address these health problems, which were reviewed for scientific merit. Revisions were sought from those proposals identified as suitable, with a request that contacts be established between each PI and the PI's of other related proposals, in order to facilitate the development of truly collaborative, multilateral networks of projects. These revised proposals were ranked by a second peer review committee, which recommended that the overall project develop modules for two of the originally identified five diseases; one for leishmaniasis and one for cystic hydatid disease. Each module was led by a scientific coordinator and designed as a multilateral network, as opposed to a strict bilateral arrangement between a single Arab and single Israeli group.

2. Objectives to be evaluated

The purpose of this program is to conduct coordinated, regionally focussed, basic and applied research in two diseases (leishmaniasis and cystic hydatid disease) which profoundly impact the people of the Middle East. The overall aim of the program is to support research leading to a better understanding of the factors influencing distribution of infection and the resulting clinical outcomes, to develop and test new intervention strategies which will contribute to the control of these diseases, and to promote peace in the region through scientific cooperation. The intent of this program is to support research which can only be performed in endemic areas, such as population- based studies and/or aspects of disease which are specific to this region. Research supported by the program will contribute to the development of disease prevention and control programs in the region, and to the development of the research capability of the applicable institutions in the area. The objectives listed in the agreement between NIAID and USAID are given below.

1. to administer and scientifically oversee a cooperative research project;

2. to characterize the epidemiology and epizoology of important vector-borne diseases from the following list: leishmaniasis, filariasis, schistosomiasis, malaria and hydatid disease;

3. to coordinate collaborative research in a network of institutions in the Middle East where scientists participate in research on one or more of a set of approved tropical disease modules;

4. to implement clinical trials to evaluate efficiency of chemotherapy, especially comparing treatment of children and adults, and symptomatic versus asymptomatic disease;

5. to study the natural history of untreated asymptomatic infection;

6. to develop strategies and implement regional control projects; evaluate costs and benefits of strategies; and

7. to develop national programs with specific goals for prevention and control.

C: PURPOSE OF EVALUATION:

The purpose of this mid-term evaluation is to determine whether or not significant progress is being made, compared to that expected, toward the stated objectives of the project and whether annual work plans were realistically and successfully implemented. It is also important to analyze the project management system; the country coordinators and the research committee, to determine to what extent work underway is reviewed and revised relative to specific objectives, how disputes are resolved, and whether funds flow to PIs in a timely manner.

Since the MERC program has a mandate to foster the Middle East peace process by funding projects of cooperation and collaboration between scientists and institutions in Israel and its Arab neighbors, specific attention is to be given to determining the nature and extent of this cooperation, and whether project activities are conducted in parallel or are truly collaborative.

EVALUATION

A. NIAID PROJECT MANAGEMENT

Project Officer: Kathryn S. Aultman, Ph.D., National Institute of Allergy and Infectious Diseases, NIH
Primary Reviewer: Brian Bock, Ph.D. USAID

The NIAID is the primary administrative institution for this project, with responsibilities which include: 1) administration of grant monies, 2) submission of technical and financial reports to AID. 3) operation of the project, 4) coordination among participating institutions in the project, and 5) periodic evaluation of project progress. The execution of these responsibilities by NIAID will be considered in this order.

Administration of grant monies. NIAID made the first disbursement of funds to the participating institutions contingent upon receipt of an acceptable 6-month work plan, and subsequent biannual disbursements contingent upon receipt of both a technical report for the previous six month period and work plan for the pending six month period. In the single instance where circumstances caused a temporary delay in the execution of one of the sub-projects, funds were withheld until evidence that progress was again underway. Thanks to this mechanism, the steady productivity of the NIAID project over its first two years has been thoroughly detailed in its regularly submitted technical reports. This disbursement mechanism appears to motivate the PIs to expeditiously circumvent unforeseen obstacles that prevent the continued progress of their projects. It also results in an explicit, objective record when conditions warrant the withholding of funds from specific sub- projects, in a manner that precludes any impressions of partiality on the part of the administrative staff. The NIAID mechanism for disbursing funds for this project has worked very well, and should be adopted as a model for other USAID MERC projects.

Submission of technical and financial reports to AID. NIAID has submitted all required technical and financial reports to AID in a timely manner.

Operation of the project. NIAID has been intimately involved in promoting the successful operation of this NIAID MERC project. For example, NIAID played a crucial role in helping the Lebanese sub-project overcome initial obstacles in procuring needed equipment and supplies, as well as helping the Moroccan sub-project establish an acceptable means for efficiently receiving funds. NIAID also played a key role as intermediary in relaying communications, supplies, and samples between Israeli and certain Arab sub-grantees (in those cases where direct interactions among them were deemed impossible or unduly risky).

The evaluation committee recognizes the desirability of incorporating a US institution such as the NIAID into MERC projects to provide just this kind of support. Often, the US institution acts as a crucial catalyst in accelerating the establishment of institutional infrastructures and collaborative precedents for the participating countries in the region. However, an equally important role of the US institution is to eventually wean the Middle East institutions from dependence on them, as a means of insuring that the regional collaboration network established during the project continues to function after the grant period has ended. The committee feels that NIAID should now attempt to promote direct communication between the sub-grantees whenever possible. Also, the role of intermediary in exchanging materials and samples between the Israelis and certain Arab collaborators should be transferred to one or more of the other Arab institutions which do not face as many obstacles in dealing directly with the Israelis.

Coordination among participating institutions in the project The NIAID has fostered excellent coordination and collaboration among the sub-grantees in this project, largely due to the emphasis that has been placed on the establishment and adherence by all participants to the common work plan. The NIAID also has been successful in promoting frequent joint meetings among the PIs, both in the Middle East and elsewhere. There also have been numerous exchanges of PIs and assistants among the collaborating laboratories, which has helped the process of standardizing methods among the different laboratories and/or increasing the level of inter-dependence among them. However, some opportunities for coordination among projects in terms of field research have been missed. For example, the Lebanese project brought in European scientists to help provide technical expertise on conducting field work with potential Leishmania vector and reservoir species, rather than solicit assistance from collaborating sub-projects. The committee recommends that NIAID be vigilant for such opportunities when reviewing future sub- project work plans. In cases where the necessary expertise is lacking in all sub-projects, an effort should be made to arrange for external assistance to be provided, but with all sub-projects invited to participate in the training.

Periodic evaluations of project progress. As mentioned previously, the NIAID mechanism of making disbursement of funds contingent upon receipt of technical reports has functioned well. However, in several instances the results presented in technical reports address topics not explicitly included in the original objectives of the project. The committee recognizes that science is by definition an unpredictable process and concedes that researchers must be assured a certain flexibility to alter their research directions in light of preliminary results or serendipitous discoveries. On the other hand, the USAID MERC Program maintains the criteria that all MERC projects must have direct relevance to development in the Middle East region, broadly defined. There is a risk that excessive re-direction of research efforts may result in perhaps quality science, but in areas that are not of a priority to the AID MERC program. In order to preclude projects from drifting too far from their stated objectives, without unduly stifling their scientific flexibility, the committee suggests that NIAID modify their policies to make disbursement of funding contingent upon receiving either 1. evidence that progress has been made towards achieving established project objectives, or 2. evidence that progress towards alternative objectives has been made, but with an additional specific justification for the shift in research emphasis and explanation for how this change still contributes towards reaching the original goals of the project.

B. COOPERATION

1: The development of the program, the initial review process, and the initial structure of the program:

As the first step in the development of the current network, a number of scientists from the Middle East were invited to a preliminary meeting to present their views of the most pressing health problems facing their countries. As the result of this meeting, five diseases were identified, based on their health and economic impact on the populations of the endemic countries. The review committee felt that the process of convening scientists from the endemic countries to identify the most important parasitic diseases was a sound one. This process met one objective of the program, which was to address problems of health importance seen by regional experts as representing the most serious threats to the health and well being of the endemic populations.

Based upon the results of this meeting, the NIAID issued a request for proposals for the NIAID-MERC program. These proposals were initially reviewed for scientific merit by a peer review committee. On the basis of this initial review, a number of scientifically sound proposals were identified. The authors of these proposals were then asked to submit a revised version of

their proposals for a secondary review. These authors were provided with the abstracts of the other projects deemed scientifically sound, and asked to describe in detail how they felt that their project might be able to collaborate with one or more of the projects from the other investigators. For example, the Israeli scientists were asked to present a work plan for a collaborative effort involving at least one Arab group, while the Arab scientists were asked to present a plan for cooperative experiments involving at least one Israeli group. The reviewers felt that this was a significant factor in the subsequent success of the project, as the authors were asked to think through plans for collaboration prior to receiving support. This process allowed the subsequent collaborations among investigators to develop much more quickly that would have been possible if the projects had been reviewed without regard to how collaborative relationships would be developed by the investigators.

The revised proposals were then ranked by a second peer review committee. At that time, the decision was made by the project staff to develop two disease modules to be supported in the final network. One of these networks was concentrated on leishmaniasis and the second concentrated on hydatid disease. The modules contained at least one Israeli group, and one or more groups from the Arab countries. The modules were thus designed as a multilateral network, as opposed to a strict bilateral arrangement between a single Arab and single Israeli group. The review committee felt strongly that this multilateral organization was the most important factor in the subsequent success of the program. The multilateral approach has also allowed the scientists involved to interact to a much greater extent, and with much greater success than would have been possible under a structure involving bilateral relationships alone.

2: The success of the NIAID MERC network in promoting collaborative and cooperative relationships among Israeli and Arab scientists:

The reviewers felt that the NIAID MERC program has proven quite successful in encouraging cooperation among the Israeli and Arab members of the network. The review committee felt that several factors were instrumental to this success. First, and perhaps the most important was the multilateral organization of the program. The multilateral focus of the program allowed the Arab scientists to interact both formally and informally with their Israeli counterparts to a much greater extent that would have been possible if the program had been organized on a strict bilateral basis. This was due to the political climate of the Middle East, where multilateral collaborations involving Israelis are considered permissible, while strict bilateral contacts carry personal and professional risks. By not exposing the investigators to these risks, the program has allowed the relationships among the Israeli and Arab scientists to develop much more fully than would have been possible if the program had mandated strict bilateral relationships.

Apart from the success engendered by the multilateral approach, several other factors have been important in supporting the cooperation in the NIAID MERC program. These include the biannual PI meetings, the workshops sponsored by the NIAID and the short term consultancies carried out to introduce the participants to new techniques to support the efforts of the program. Perhaps the most important of these has been the workshops sponsored by the NIAID, and more recently by the MERC network members themselves. These workshops have fostered the use of common reagents, laboratory protocols and data analysis methods among the participating members. This has assisted the members of the MERC program in sharing the results of their work, as the reagents and methods are common among the laboratories.

The MERC program to support collaboration among Israeli and Arab scientists has had concrete results. The program has resulted in Israeli and Arab laboratories sharing samples and

reagents. These have involved parasite isolates, human and animal sera, sandfly reference specimens, monoclonal antibodies and parasite antigens. The exchanges have been both from Israeli laboratories to Arab laboratories and from Arab laboratories to Israeli laboratories. Furthermore, there have been many instances of visits carried out by Israeli scientists to Participants in the Workshop on Parasite Identification, Casablanca, Morocco, March 1995 cooperating Arab laboratories, and *vice versa*. These have all served to strengthen the cooperative efforts in the region. Two instances of such collaborative efforts were noted by the review committee as perhaps the most significant examples of collaboration between Israeli and Arab scientists. The first of these was a multi center study carried out by Israeli and Arab scientists to evaluate the coproantigen assay for the detection of *Echinococcus spp.* infection in dogs. The second was a collaborative workshop on the monoclonal antibodies for the identification of *Leishmania*. This workshop, jointly organized by Drs. Charles Jaffe of Hebrew University and Dr. Nouzha Guessous of Hassan II University in Casablanca, was held in Casablanca. It involved 19 individuals from all of the *Leishmania* laboratories in the network, as well as several external participants supported by the WHO. The workshop resulted in a publication of a manual, which was jointly authored by the Israeli and Moroccan scientists. The network has also resulted in the joint publication of a number of abstracts of presentations at international meetings. Finally the network members are currently preparing a review article on the epidemiology of leishmaniasis to be co-authored by the Israeli and Arab members of the network.

In summary, the review committee was impressed by the extent to which the NIAID MERC program has succeeded in fostering collaborative relationships among the Arab and Israeli members of the program. The reviewers ascribed this success primarily to the multilateral structure of the program, and to the standardization of reagents and methods supported by the workshops carried out by the members and by the NIAID.

C. TECHNICAL PROGRESS

Sub-Project 1: Ecology of Leishmaniasis in the North of Morocco
Principal Investigator: Dr. Nouzha Guessous Idrissi, Faculte de Medicine et Pharmacie, Universite Hassan II, Casablanca, Morocco
Primary Reviewer: Dr. Philip Lawyer

Background. Visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) are important health and economic problems in Morocco. VL due to *Leishmania infantum* appears to be hypoendemic with sparse foci in northern Morocco. Its distribution correlates well with humid and sub-humid regions of the Atlantic and Mediterranean coastal plains. CL due to *L. major* occurs in epidemic foci in pre-Saharan zones south of the Atlas and Anti-Atlas mountains. CL due to *L. tropica* is hypoendemic, occurring in semi-arid mountainous regions between Tadla (northeast) and Agadir (southwest). Until 1993, the government depended on extramural research groups for help in solving their health problems. No national system for reporting leishmaniasis cases had been established and no indigenous Moroccan research teams worked on these diseases. Ecoepidemiological surveys commissioned or sponsored by the Ministry of Health in the 1980s and early 1990s were conducted by French researchers with little or no indigenous collaboration. These surveys focused on epidemic CL (*L. major*) in southern Morocco and hypoendemic CL (*L. tropica*) in west-central Morocco. Epidemiological investigations on VL and CL in the northern part of the country were disregarded. Basic data (identification of parasites, vectors and reservoirs) were usually collected in country and taken to France where they were assimilated and published. Consequently, virtually all the literature on leishmaniasis in Morocco is of French origin. Dependence on foreign sources for solutions did nothing to build long-term infrastructure or multidisciplinary research teams within the country and did not foster regional cooperation. The aim of the current project is to form a Moroccan-led multidisciplinary research group that will contribute to and reinforce a national leishmaniasis control program and, through collaboration with other MERC partners, contribute to the control of leishmaniasis on a regional basis. Two complementary, multidisciplinary teams are engaged: one from the Faculty of Medicine of Casablanca (Faculte de Medecine et de Pharmacie de Casablanca) and one from the Agronomic and Veterinary Institute of Rabat (Institut Agronomique et Veterinaire Hassan II de Rabat). The specific objectives are:

1. to identify regions of risk of visceral, canine and cutaneous leishmaniasis in the North of the country;

2. to study the epidemiological mechanisms of leishmaniasis transmission (vectors, man, reservoirs) in the identified foci;

3. to develop animal models for maintaining *Leishmania* strains isolated from the field, and initiate immunological studies.

Detailed Scientific Review:

Objective 1: To identify regions of risk of visceral, canine and cutaneous leishmaniasis in the North of the country. The wording of this objective is confusing, since leishmaniasis in canines due to *L. infantum* is also visceral. This objective was mostly accomplished during the first year and a half of the study. The approach consisted of a retrospective survey of Ministry of Health and Ministry of Agriculture records of human and canine leishmaniasis cases reported in the northern part of

the country between 1991 and 1993, and a passive case detection survey of regional and national health facilities in 1994.

A retrospective study of the archives of the Ministry of Health, hospital records from the University Hospitals of Casablanca and Rabat, and personal contacts with regional pediatric and veterinary service providers in the 13 defined regions of the project revealed 75 human visceral and 16 human cutaneous leishmaniasis cases between 1991 and 1993 in the northern part of Morocco. Most of these were from the Khemisset-Meknes-Taounate axis. Canine cases identified were among hunting dogs, making geographic localization difficult. Contacts in both human and animal health services were made in the regions of Khemisset, Tetouan, Meknes, Taza, and Taounate. As part of the passive case detection survey of regional and national health facilities, data were collected from the University Ibn Rochd Hospital of Casablanca (CHIR) and from the Parasitic Disease Control Service of the Ministry of Health. In 1995 sixty cases of human visceral leishmaniasis were reported to the Ministry of Health, of which 58 were from the north. One hundred sixty-one cases of human cutaneous leishmaniasis were reported of which 157 were from the south. In an active field survey conducted in the locality of Hrira, Khemisset Province, one man was found with a CL lesion on his forearm.

As a result of these initial surveys, and at the recommendation of MERC colleague, Dr. Shaden Kamhawi of Jordan, who visited several of the case sites, three representative foci were selected for field work. These include Khemisset Province, a totally zoonotic focus of VL in dogs, Taounate Province, a focus of human VL, and Taza, known to be a human VL focus, where recently more than 50 cases of human CL due to *L. tropica* were found in an active field survey. The Khemisset focus lies within the coastal plain at about 400 m elevation and has a typical Mediterranean climate. The Taounate and Taza foci are situated in the foothills at opposite ends of the Atlas Mountains at about 1,100 m and have semi arid climates.

Objective 2: To study the epidemiological mechanisms of leishmaniasis transmission (vectors, man, reservoirs) in the identified foci. Activities associated with this objective comprise the bulk of the laboratory and field work of the project. The start-up phase, which consumed most of the first year of the project, included creation and outfitting of a new laboratory unit ("Unite d'Etudes et de Recherche sur les Leishmanioses") at the Faculte de Medecine et de Pharmacie de Casablanca. This laboratory unit contains two subunits: (1) culture and identification of *Leishmania* strains and (2) entomology. The primary investigator of each subunit was sent for specialized training to MERC partner laboratories where expertise was available. Dr. Myriam Riyad visited the Pasteur Institute in Tunis, Tunisia, to learn from Dr. R. Ben Ismail techniques for culture and cryo-preservation of *Leishmania* parasites and isoenzyme typing. Dr. Abdelaziz Hamdani visited the Entomology Laboratory at Yarmouk University to be trained by Dr. Shaden Kamhawi in entomological methods including collecting, mounting, identification and dissection of sand flies. Prof. Guessous-Idrissi, the project principal investigator, also visited these two MERC partner laboratories to discuss laboratory organization and technical points.

Parasite culture and typing. Parasite culture techniques were standardized using reference strains donated by the Institut Pasteur de Tunis. Once these procedures were worked out, the team began culturing isolates from suspected cases in humans and dogs. Culture success rate so far has been about 33% (43/127). The low success rate was attributed to contamination and lack of experience. This should improve with experience and with improvements in laboratory facilities, equipment and reagents. Parasite characterization procedures using monoclonal antibodies (MABs)/ELISA, excreted factor (EF) and isoenzyme analysis (cellulose-acetate electrophoresis)

were developed for use in both the Casablanca and Rabat laboratories. Prof. Charles Jaffe of the Hadassah Medical Center, Hebrew University, Jerusalem, Israel, visited the Casablanca and Rabat laboratories in December of 1994 and demonstrated *Leishmania* identification methods using monoclonal antibodies and excreted factors. While there he also helped lay the ground work for the regional workshop on monoclonal antibody techniques jointly sponsored by the Moroccan and Israeli teams in Casablanca in March of 1995. The laboratory now has the capability to identify cultured isolates using MABs, EF's and isoenzyme analysis. A battery of three enzyme systems recommended by Dr. R. Kreutzer are being tested for species identification and four additional enzyme systems for zymodeme assessment. The research team felt that additional training on site by a visiting expert would be useful.

Seroepidemiological surveys. An immuno-fluorescent antibody test (IFAT) and an ELISA, both using local strains of *Leishmania,* were set up for use in screening dog and human populations for seroprevalence of *Leishmania* antibody. These assays were found to be more sensitive than commercial assays used by the hospital, presumably due to the use of local strains. Based on the results of retrospective and passive detection surveys, active seroepidemiological surveys were organized around index case sites in the three foci mentioned above. In a total of 14 field trips, 100 serosurveys and 197 skin tests in humans were conducted in the foci of Khemisset (canine VL), Taounate (human VL) and Taza (human CL). These identified 31 active human cases of leishmaniasis for follow up from the Taounate and Taza foci.

Surveys of canine populations included 66 field trips involving 628 dogs on which 421 clinical exams and 337 serodiagnostic tests were performed. Fifty-five dog cases were followed up. In Khemisset city and in the 6 surrounding localities no human cases were found. Seropositivity in dogs ranged from 0-27.4%, with an average of 15.8%. A follow-up survey in Sidi El Ghandour conducted in 1995 indicated seroprevalence of 28.4%. This relatively high canine seroprevalence is consistent with foci of leishmaniasis due to *L. infantum.* In the village of Ouled Hssein, in Taounate Province where human VL due to *L. infantum* occurs sporadically, canine seroepidemiology surveys showed greater than 38% seropositivity in dogs. A follow-on human seroepidemiology survey was conducted near an index case of human VL due to *L. infantum* in Taounate Province. Of 80 people (44 children) in 20 families tested by IFAT, all were seronegative and asymptomatic. Interestingly, the only *Leishmania* isolate obtained from Taounate Province was from a dog and was typed as *L. tropica*. In Taza the team expected to find VL due to *L. infantum,* since 23 cases had been reported from there between 1991 and 1995. They were surprised to find four cases of CL, isolates of which were typed as *L. tropica.* They have since found a total of 30 CL cases in Taza, all due to *L. tropica.* This is a new phenomenon for the area and is consistent with a zoonosis. It is somewhat reminiscent of *L. tropica* foci in northern Jordan and in Israel. The village of Taza is situated on a hilltop. Most of the CL case sites abut the surrounding valley, which serves as a garbage dump for the village and offers plentiful natural and man-made habitat for vectors and small mammals.

The results of these surveys prompted a request by the Ministry of Health for a seroepidemiology survey of the whole of Taounate Province. Since *Leishmania* serology by ELISA is more suitable for large sampling, the team plans to use this method in large surveys in Taounate and Taza.

Vector surveys. Sand fly surveys are being conducted in all three foci. A total of 22,000 sand flies representing 10 species were collected from urban and rural habitats in these areas between 16 May 1994 and 24 November 1995. Collection methods included sticky (oiled) traps, CDC light traps and aspirators (resting site collections). One infected *Phlebotomus ariasi* was collected

Taounate focus. The parasites were typed as *L. infantum* using monoclonal antibodies. In the Khemisset area, the three predominant vector sand fly species collected were *P. sergenti* (48%, vector of *L. tropica*), *P. perniciosus* (23%, vector of *L. infantum*) and *P. longicuspis* (20%, vector of *L. infantum*). Other known vectors included *P. ariasi, P. langeroni, P. longicuspis, P. pernisciosus*, all vectors of *L. infantum*, and *P. papatasi*, the usual vector of *L. major.* The sand fly fauna of Taounate differed from that of Khemisset in that *P. pernisciosus* was found in very low numbers (<1% of total) and *P. papatasi* was absent. In Taza, *P. longicuspis* and *P.sergenti* were the two predominant species (46% and 40.5%, respectively), followed by *P. ariasi*, *P. papatasi* and *P. pernisciosus*. The density of sand fly populations in all three foci showed two peaks, June and September. The June peak was due to populations of *P. sergenti* (vector of *L. tropica*) and the September peak to populations of *P. pernisciosus* and *P. longicuspis* (vectors of *L. infantum*).

To date, sand fly collections have focused more on diversity and relative and seasonal abundance of species than on vector incrimination and parasite-sand fly-man interactions. There is a tendency to assume that the peak transmission season for leishmaniasis corresponds to periods of peak density of sand fly populations. This may or may not be true, depending on parasite infection rates in the vectors and vector interactions with their hosts. It is not uncommon to find the highest rate of infection in the vector during the period of lowest population density. To obtain a true perspective of transmission parameters, the team should focus on sand fly-man/reservoir interactions at specific case sites using human and animal-baited collections. Collection and dissection of manbiters through the sand fly season will reveal parasite infection rates and enable calculation of the entomological inoculation rate (the rate at which a susceptible host is fed upon by sand flies with mature leishmanial infections). The entomology team plans to continue sand fly surveillance in the Khemisset and Taounate foci and will expand to include the recently discovered *L. tropica* focus in Taza. The entomology team has analyzed a small sample of sand fly bloodmeals collected in Taza and plan to expand this to other foci. Special training for this effort was provided in Tunis by Dr. H. Louzir of the Pasteur Institute. Sand fly bloodmeal analyses will enable calculation of human biting indices (the proportion of bloodmeals a particular vector takes from humans) and will help narrow the list of potential reservoirs.

Reservoir studies. Based on the results of the seroepidemiological surveys, canines are the presumed reservoirs of visceral *L. infantum* in Morocco. It is not known whether they are also reservoirs for cutaneous *L. infantum* in Morocco. There have been reports from Italy of rodent reservoirs for cutaneous *L. infantum.* A rodent survey was scheduled for the Khemisset area but has not been implemented. Leishmaniasis due to *L. tropica* does not occur in the Khemisset area in spite of the presence of a suitable vector (*P. sergenti*). This suggests that a suitable reservoir is absent. Both VL due to *L. infantum* and CL due to *L. tropica* occur in Taounate province. Again, dogs are the presumed reservoirs of the visceral *L. infantum,* but the only confirmed canine leishmaniasis in Taounate province was a visceral case due to *L. tropica*. Whether canines serve as reservoirs for both *Leishmania* species, or whether there are other reservoir animals involved remains in question. The reservoir of cutaneous *L. tropica* in Taza is unknown. The team plans future field studies to identify reservoirs in Taounate and Taza.

Objective 3: To develop animal models for maintaining *Leishmania* strains isolated from the field, and initiate immunological studies. The ultimate goal of this objective is to identify a vaccine candidate.

Nine local dogs, 6-12 months old and free of *Leishmania* and other parasites were inoculated intravenously with amastigotes of one of two local canine strains, *L. infantum* (LR) from

Khemisset (4 dogs) and *L. tropica* (T10) from Taounate (5 dogs). Amastigotes were obtained from organs of infected dogs that had been cryopreserved intact. To insure that the parasites remain viable during cryopreservation, it would be better to harvest them from the organs and then cryopreserve them in a cryprotectant such as glycerol or DMSO. This will also facilitate verification of viability and standardization of the inoculum. All nine dogs developed visceral infections and were followed up with blood collection (for serology, cellular immunology and parasite culture) and monitoring of physical signs. All nine dogs died within 55 weeks and were autopsied and parasite cultured. These results differ from published data suggesting that laboratory-infected dogs usually self cure and are not a good model for natural infection. A possible explanation is that the Rabat team used amastigotes harvested from infected organs rather than cultured promastigotes used by other workers.

Concurrently, the clinical status of 13 naturally infected dogs from Khemisset (*L. infantum*) area was followed. These animals showed fever, tachycardia, tachypnea, cachexia, alopecia, onchogryphosis, superficial lymph node hypertrophy, ocular lesions, and skin lesions. At necropsy they showed lymphadenopathy, splenomegaly, hepatomegaly, lymphopenia, hypergammaglobulinemia and proteinuria. A skin test, using an *L. infantum* Mon-1 was also employed for comparison with seropositivity in naturally infected dogs in the Khemisset area. This was later abandoned due to highly variable and weak response in seropositive dogs.

An immunization trial was initiated in five dogs that were free of *Leishmania.* Three of the dogs were injected with crude antigen (*L. infantum* LR) encapsulated in liposomes. Two dogs received only empty liposomes. At the time of this review, none of the dogs had been challenged. The three immunized dogs showed an antibody response 6-10 days after the primary immunization which peaked 20 days post primary immunization. A secondary immunization amplified the antibody titer. Preliminary results of lymphocyte transformation tests on cells from experimentally infected dogs and immunized dogs showed some specific stimulation in response to either crude antigen or gp63 (from Charles Jaffe). Cells of control-group animals showed no response.

The groundwork was laid for later study of cellular response to leishmaniasis in naive, sick and recovered dogs. The Rabat team plans to continue the study of physiopathology of leishmaniasis in naturally and experimentally infected dogs, as well as the immunological parameters. They will continue to evaluate the immune response levels in immunized dogs (unchallenged) using crude antigen encapsulated in liposomes.

Summary: Of the MERC projects reviewed, the Moroccan project was the most comprehensive and most impressive. Under the direction of Prof. Guessous Idrissi, two new laboratories, one in Casablanca and one in Rabat have been established and two complementary and multidisciplinary teams have been trained. The Moroccan teams have essentially accomplished their first objective and significant portions of the second and third objectives. It is reasonable to expect they will accomplish the remaining portions of objectives two and three by the end of the project's third year.

The first objective was accomplished through retrospective surveys of hospital records, passive and active case-finding surveys. Three diverse foci were identified and selected for extensive field work. These include Khemisset Province, a totally zoonotic focus of VL in dogs, Taounate Province, a focus of human VL, and Taza, thought to be a human VL focus, but where several cases of human CL due to *L. tropica* were recently found.

Preparations to accomplish the second objective consumed most of the first year and involved creating and outfitting new laboratories at the Faculte de Medecine et de Pharmacie de Casablanca and at the Institut Agronomique et Veterinaire Hassan II de Rabat, and in training investigators in the techniques appropriate to accomplish the work. The laboratories have now established good capabilities for characterizing/identifying cultured isolates using MABs, EF's and Isoenzyme analysis.

Based on the results of the retrospective and passive detection surveys, active seroepidemiological surveys were organized around index case sites in the three foci mentioned above. In Khemisset city and in the 6 surrounding localities no human cases were found. Seropositivity in dogs ranged from 0-27.4%, with an average of 15.8% and 55 infected dogs were identified for follow up. In the village of Ouled Hssein, in Taounate Province, canine seroepidemiology surveys showed greater than 38% seropositivity in dogs. Of 80 people (44 children) in 20 families tested by IFAT, all were seronegative and asymptomatic. Interestingly, the only *Leishmania* isolate obtained from Taounate Province was from a dog and was typed as *L. tropica*. In Taza, the team was surprised to find more than 30 cases of CL due to *L. tropica.* Distribution of the cases suggests that this outbreak is a zoonotic.

A total of 22,000 sand flies representing 10 species were collected from urban and rural habitats of all three foci between 16 May 1994 and 24 November 1995. Collection methods were limited to sticky traps, light traps and aspirators. One *Phlebotomus ariasi* was collected in Taounate focus infected with *L. infantum*. The density of sand fly populations in all three foci showed two peaks, June and September. The June peak was due to populations of *P. sergenti* (vector of *L. tropica*) and the September peak to populations of *P. perniciosus* and *P. longicuspis* (vectors of *L. infantum*). To date, sand fly collections have focused on species diversity and relative and seasonal abundance rather than on vector incrimination and parasite-sand fly-man interactions. The entomology team plans to continue sand fly surveillance in all three foci, to include bloodmeal analysis.

Canines are the presumed reservoirs of visceral *L. infantum* in Morocco. It is not known whether they are also reservoirs for cutaneous *L. infantum.* Leishmaniasis due to *L. tropica* does not occur in the Khemisset area in spite of the presence of a suitable vector (*P. sergenti*), suggesting that a suitable reservoir is absent. Both VL due to *L. infantum* and CL due to *L. tropica* occur in Taounate province. Dogs are the presumed reservoirs of the visceral *L. infantum.* However, the only confirmed canine leishmaniasis in Taounate province was a visceral case due to *L. tropica*. Whether canines serve as reservoirs for both *Leishmania* species, or whether there are other reservoir animals involved remains in question. The reservoir of cutaneous *L. tropica* in Taza is unknown. The team intends to conduct surveys for potential reservoirs in Taounate Province and in Taza.

Nine local dogs, 6-12 months old and free of *Leishmania* and other parasites were inoculated IV with one of two local canine strains, *L. infantum* (LR) from Khemisset (4 dogs) and *L. tropica* (T10) from Taounate (5 dogs). All nine dogs died within 55 weeks in contrast to published data suggesting that laboratory-infected dogs usually self cure and are not a good model for natural infection. A possible explanation is that the Rabat team used amastigotes harvested from infected organs rather than cultured promastigotes used by other workers. Concurrently, the clinical status of 13 naturally infected dogs from Khemisset (*L. infantum*) area was also followed. These animals showed fever, tachycardia, tachypnea, cachexia, alopecia, onchogryphosis, superficial lymph node hypertrophy, ocular lesions, and skin lesions. At necropsy they showed lymphadenopathy,

splenomegaly, hepatomegaly, lymphopenia, hypergammaglobulinemia and proteinuria. A skin test was employed for comparison with seropositivity in naturally infected dogs in the Khemisset area

An immunization trial using crude antigen (*L. infantum* LR) encapsulated in liposomes was initiated in a group of laboratory dogs. The immunized dogs showed antibody response 6-10 days after the primary immunization which peaked 20 days post primary immunization. A secondary immunization amplified the antibody titer. Preliminary results of lymphocyte transformation tests on cells from experimentally infected dogs and immunized dogs showed some specific stimulation in response to either crude antigen or gp63. Cells of control-group animals showed no response.

Collectively, the Moroccan teams have given 26 oral or poster presentations at national, regional and international scientific meeting, co-authored one technical handbook with Israeli partners, published one manuscript (Revue Marocaine de Medecine et Sante), submitted five manuscripts (Am. J. Trop. Med. Hyg, Trans. R. Soc. Trop. Med. Hyg., Parasite), completed two veterinary masters theses and initiated 5 Doctor of Science theses.

Recommendations from the committee are as follows:

1. Increase the number of enzyme systems used for identifying *Leishmania* parasites by isoenzyme electrophoresis. Seven to 12 enzyme systems might be optimal. Refer to Mebrahtu et al., 1992. Biochemical characterization and zymodeme classification of *Leishmania* isolates from patients, vectors, and reservoir hosts in Kenya. *Am J Trop Med Hyg 47* : 852-892.

2. Participate with other MERC partners to develop a standardized, sensitive skin test using local antigens that can be used consistently throughout the region to screen large human and animal populations.

3. Employ all-night human- and animal-bait to collect manbiters and develop vector biting activity profiles.

4. When dissecting female sand flies, first determine the reproductive age (nulliparous, parous, multiparous) by examining the accessory glands for opaque granules and/or the ovaries for follicular dilatations. It is necessary only to examine the guts of parous and multiparous females for flagellates to determine the parasitological infection rate in the vector.

5. Harvest amastigotes from infected organs of laboratory animals by disrupting the tissues in a tissue grinder and centrifuging to separate the parasites. Cryopreserve the parasites for future use. Prior to use, count the viable amastigotes on a hemacytometer so that the inoculum can be standardized.

Efforts Toward establishing regional collaborations: The Moroccan team probably has done more to foster cooperation with other MERC Program participants and to support the common work plan than any other participant. They have availed themselves of the special expertise of various other MERC laboratories for training their own investigators, exchanged isolates, reagents and technologies, and have invited collaborations with Israeli and other Arab teams to accomplish their program objectives. They have actively participated in semi-annual PI meetings, co-sponsored and hosted a regional training workshop on use of MABs and EF's for *Leishmania* typing, and participated in regional workshops hosted by other MERC partners. Significant cooperative activities are as follows:

- Dr. Myriam Riyad visited the Pasteur Institute in Tunis, Tunisia, to learn from Dr. R. Ben Ismail techniques for culture and cryo-preservation of *Leishmania* parasites and isoenzyme typing.

- Dr. Abdelaziz Hamdani visited the Entomology Laboratory at Yarmouk University to be trained by Dr. Shaden Kamhawi in entomological methods including collecting, mounting, identification and dissection of sand flies.

- Prof. Guessous-Idrissi, the project principal investigator, also visited these two MERC partner laboratories to discuss laboratory organization and technical points.

- Mr. A. Hamdani and Mr. A. Essari received training in sand fly bloodmeal identification from Dr. H. Louzir of the Pasteur Institute, Tunis.

- Prof. Charles Jaffe of the Hadassah Medical Center, Hebrew University, of Jerusalem, was invited to the Casablanca and Rabat laboratories in December of 1994 to demonstrate *Leishmania* identification methods using monoclonal antibodies and excreted factors. While there he and the PI laid the ground work for the joint Israel-Morocco sponsored regional workshop on monoclonal antibody techniques.

- The WHO/MERC International Workshop on *Leishmania* Typing Using Monoclonal Antibodies and Excreted Factors was hosted in Casablanca by the PI and co-sponsored with Prof. Charles Jaffe of the Hebrew University of Jerusalem. A joint technical handbook was prepared.

- Purified gp63 antigens were obtained from Prof. Charles Jaffe, Hebrew University of Jerusalem for use in developing a dog-*Leishmania* model, in studying the immune response and in efforts to validate a vaccine.

- Monoclonal antibodies were obtained from Prof. Jaffe and expertise in excreted factor typing from Dr. Lee Schnur, Hebrew University of Jerusalem.

- Dr. Shaden Kamhawi of Yarmouk University, Jordan, was invited to visit several of the case sites in Morocco to provide training in sand fly collection and to help identify suitable field study sites.

Sub-Project 2: Comprehensive Approach for the Prophylaxis of Leishmaniasis
Principal Investigator: Charles Jaffe, Ph.D. Hebrew University, Jerusalem.
Primary Reviewer: Franklin A. Neva, M.D.

Background: Despite its title, this project deals solely with visceral leishmaniasis in Israel. It is broken down into the following components, according to the work plan starting in February 1994:

1. Serum samples that have been sent to Carmel Hospital in Haifa for routine analyses will be tested for antibodies to *L. donovani* crude antigen by ELISA test. The serum samples would be selected to represent nine settlements in northern Israel, including regions considered both endemic as well as non-endemic for leishmaniasis. [The number of settlements reported in Technical Report #1 was 24.]

2. A similar serodiagnostic survey of dogs and people in Tamra will be conducted from blood specimens collected on filter paper. Tamra is the area for the echinococcosis study being

conducted by Dr. El-On, so the filter paper specimens are collected in duplicate; one for leishmanial and the other for hydatid serology.

3. Leishmanial antigens gp70, dp72 and gp63 will be purified. Dp72 will be used as a vaccine candidate antigen in mice; gp70 as a possible diagnostic antigen for early infection, and all antigens to follow antibody response in patients after drug therapy of visceral leishmaniasis.

4. Assays to examine production and expression of cytokines by tissues of BALB/c mice will be conducted under various conditions of infection with *L. donovani*.

Detailed Scientific Review:

There are a number of discrepancies between the objectives of this research project as originally outlined in the proposal, and the results as reported in the technical reports. Some examples of these discrepancies are listed below:

- Cloning of parasite antigens for diagnosis of visceral leishmaniasis is first mentioned in Work Plan No. 3 for February 1, 1995 to July 17, 1995. But this was already done and reported in technical report No. 1 for February 18, 1994 to August 17, 1994, a year earlier.

- The intention to use PCR for analysis of filter paper blood samples from dogs in Tamra for visceral leishmaniasis is described in Work Plan No. 2 for August 18, 1994 to February 17, 1995. But no mention of results can be found in any of the three technical reports.

- Results of ELISA assays for detection of leishmanial parasites in sand flies is reported in Technical Report No. 3, but plans to carry out this type of work are not mentioned in any of the four Work Plans.

- Although the evidence for existence of glycolipid anchors on gp70 and dp72 leishmanial proteins (as described in Technical Report No. 1) and their localization in the parasite by immunogold staining (Technical Report No. 3) are of basic scientific interest, such information is not really needed to achieve objectives of this project. [Editor's note: These were a portion of the initial proposal upon which the contract was based.]

- It might also be noted that even though cytokine studies in BALB/c mice under various conditions of leishmanial infection were spelled out as specific research objectives, such basic scientific investigations are somewhat removed from the more applied research objectives of the other MERC projects.

In spite of the inconsistencies between stated research objectives and the work reported, the project has been productive as evidenced by the following results:

1. The system of screening serum samples from scattered communities in northern Israel has detected presumptive evidence of low level transmission of visceral leishmaniasis. By the end of the third reporting period there were twenty-one of 1034 (2.03%) serum samples from the endemic regions which were positive against crude leishmanial antigen.

2. Evidence for a new focus of transmission of leishmaniasis in dogs (8 out of 60) and one human case was found recently in Mattiyahu, a settlement only twenty minutes to the west of Jerusalem.

3. Several of the forty sera from rodents collected near Kafr Adumim, a settlement near Jerusalem, were positive for anti-leishmanial antibodies. This is a new focus of *L. tropica* at roughly the same latitude as a new focus of *L. tropica* in the mountains of Jordan.

4. An assay for identification of leishmanial parasites in sand flies using species-specific monoclonal antibodies was developed. It can be used as either a dot blot or an ELISA test and will detect down to four thousand organisms. Preliminary data suggest that this degree of sensitivity will identify infected sand flies collected in the field.

5. In infected dogs, antibody responses to a purified recombinant antigen, dp72, could be demonstrated early - within 2-3 weeks. This antigen, therefore, may be helpful in diagnosis of early visceral infections, before obvious disease develops.

Even though the main effort in the Tamra area of northern Israel is related to the hydatid disease project of Dr. El-On, the arrangement of providing duplicate filter paper blood samples to screen for evidence of leishmanial infection is working very well. It has already provided evidence of human infections in the area.

The usefulness of the collection of serum samples that come to Carmel Hospital in Haifa from various settlements has yet to be determined. However, the availability of such serum samples might prove to be very useful in mapping the existence of other infections of diseases besides leishmaniasis or hydatid disease. For example, presence and distribution of viral hepatitis, or zoonotic infections such as brucellosis could be determined.

The interaction between the Israeli investigators and the local population of the Tamra area, which has developed as a consequence of the project, was very positive. The fact that the project involves diverse ethnic and cultural groups - people from Israeli, Arab and Druse settlements - is unique. Our site visit showed that the cooperation between the local health care system and the Israeli investigators was very good. Hopefully, this spirit of cooperation can be extended to dealing with other public health problems.

Efforts towards establishing regional collaborations: Dr. Jaffe has been one of the most successful MERC program PIs in establishing collaborative arrangements with other members of the MERC program. In pursuit of this objective:

1. Professors Jaffe of Israel and Guessous Idrissi of Morocco jointly organized and sponsored a workshop on the use of monoclonal antibodies and excreted factor for identification of leishmanial parasites. Dr. Jaffe traveled to Morocco in December 1994 to prepare for the workshop; during that visit he trained the entire Moroccan team in all of the techniques to be used. The workshop itself was held in Casablanca in March 1995 and was attended by twenty participants from the five MERC countries, as well as some from Pakistan, Iran, the Sudan, Turkey, and India.

2. A five week visit in July-August, 1995 of Saadia Lasri of Morocco was hosted by Professor Jaffe. Dr. Lasri works on immunology of canine leishmaniasis and she came to learn a variety of techniques, including preparation of leishmanial antigens to be used for immunologic techniques, cell-mediated proliferation tests, Western blotting, culturing and freezing of cells, etc.

3. A two week visit by Dr. Mulkiye Kasap from the University of Cukurova in Adana, Turkey was co-hosted by Professors Jaffe and Schlein in September 1995. Dr. Kasap brought several isolates of *Leishmania* to be typed (they turned out to be *L. tropica*) and learned techniques of collecting and identifying sand flies.

Sub-Project 3: The Epidemiology of Cutaneous Leishmaniasis in North Jordan
Principal Investigator: Shaden Kamhawi, Ph.D. Yarmouk University, Irbid, Jordan
Primary Reviewer: Brian Bock, Ph.D.

Background: Cutaneous leishmaniasis is endemic in Jordan. The classical picture to emerge from studies in southern Jordan and the Jordan Valley region is that *Leishmania major* serves as the parasite species, the colonial rodent *Psammomys obesus* is the principal reservoir, and the sandfly *Phlebotomus papatasi* acts as the vector of transmission to humans. This particular rodent reservoir is restricted to semi-arid habitats which support its principal food source, salt bushes of the family *Chenopodiacea.*

Cutaneous leishmaniasis also has been reported from a completely different biotope in North Jordan, in a region with a sub-humid Mediterranean climate which supports woody vegetation and cultivated olive groves. The typical *Leishmania major* reservoir species *Psammomys obesus* does not occur in this area. Preliminary studies of the sand fly fauna of this region have demonstrated the presence not only of *Phlebotomus papatasi*, but also of several congeneric species known to serve as vectors for other species of *Leishmania.* Finally, lesions expressed by humans in this area are smaller but longer healing than cutaneous leishmaniasis lesions more typical of the foci in the more arid regions of the country, where *Leishmania major* is the known or presumed parasite. Isolation of the parasite from lesions in North Jordan also has proven difficult, in contrast to the usual ease with which *Leishmania major* is isolated in other areas of Jordan.

This evidence all implies that the cases of cutaneous leishmaniasis reported from the focus in North Jordan are not likely to be caused by *Leishmania major.* While the endemicity of cutaneous leishmaniasis in North Jordan seems low, this impression may merely be an artifact of poor health reporting in this area. Thus, a detailed study of cutaneous leishmaniasis there seems warranted. Such a study has the concomitant advantage of raising awareness of both cutaneous and visceral leishmaniasis among local health professionals and the general population, and may contribute to the documentation of visceral leishmaniasis in Jordan, as well as the eventual development of an effective control program for this unique focus of cutaneous leishmaniasis.

To meet the goal of conducting a comprehensive study of the epidemiology of cutaneous leishmaniasis in North Jordan, the following specific objectives for this project were identified:

1. To determine the true prevalence and annual incidence of cutaneous leishmaniasis in the North Jordan focus.
2. To isolate and characterize the *Leishmania* species from human cases in this focus.
3. To identify possible vectors of cutaneous leishmaniasis in the focus and attempt to incriminate one or more of the suspected vectors.

Detailed Scientific Review: During the past two years, the research team has documented a total of 72 cases of cutaneous leishmaniasis from the Bani Kinana District in North Jordan, with 75% of the cases originating from the two villages of Malka and Um-Quais. A concentration of cases along the outskirts of the villages, the fluctuation in annual incidence of cases documented, and the diffuse distribution of the known cases all suggest to the Principal Investigator that

transmission of the disease in this focus is zoonotic. Over 700 skin tests provided data for the production of an age profile for the two villages with the highest prevalence, which further suggested that the disease has been endemic in this area for some time.

The team successfully isolated and cultured *in vitro* the *Leishmania* parasite from several human cases in this area. Starch gel electrophoresis of cultured isolates confirmed that the species of parasite in this area of Jordan is *Leishmania tropica*, and not *L. major*. Comparison of isozyme profiles with isolates of *L. tropica* from Syria and Israel, as well as reference strains from the Medical Ecology Laboratory in Montpelier, France, have confirmed that the *L. tropica* parasites isolated from North Jordan pertain to a unique zymodeme, and further electrophoretic characterization of this strain is planned so that the strain may be included in the WHO Leishmania reference center collection in Montpelier.

Finally, the team has conducted extensive studies of the sand fly fauna in this area. Six possible vector species have been identified and their relative abundances in differing microhabitats have been documented. A well executed longitudinal stratification study demonstrated that absolute and relative species abundances change with time in this region, with the greatest sand fly concentrations occurring in the lowest areas of the valleys and along their rims. ELISA tests of the blood meals of captured flies indicated that some sandfly species were primarily anthropophilic, others primarily zoophilic, and others non-discriminating. To date, efforts to identify *Leishmania* parasites from within dissected female sand flies have proven unsuccessful, but efforts towards incrimination of the vector species are to be intensified in the coming field season.

Summary During the first two years of this project, the research team has already met their first two objectives (documenting the incidence of Leishmania in this area and characterizing the species involved). They also have made significant progress towards achieving their third objective (identifying possible vectors and incriminating the principal vector for *Leishmania tropica*). It is commendable that the project intends to not only continue its initially proposed activities, but also will expand its objectives during the final year. During the sand fly season, efforts will be intensified to obtain infected females and any cultures obtained will be probed using PCR techniques. Furthermore, an intensive mammal trapping effort will be conducted following the sand fly season in an effort to identify the reservoir species in this area.

Experiments on the susceptibility of local mammal species to *Leishmania tropica* infection, using captive animals, also will be conducted. Investigators from this research team are also continuing their monitoring and pilot control work on the *Leishmania major* focus from the Jordan valley region (initial studies in this area were supported by another donor). The continued involvement by team members in the Jordan valley seems particularly appropriate, given the close proximity of the Israeli Jordan valley foci there.

The committee feels that the investigators have conducted exemplary field work and solid laboratory analyses in the execution of this project to date. They seem to have a clear grasp of the important questions that remain and show every indication that they will address these questions in the coming field season. The project has excelled in providing training for students, both from Jordan and throughout the Middle East. The publication and presentations arising from this project seem adequate considering the time the project has been underway. The results obtained to date and those anticipated should have substantial relevance to the control of cutaneous leishmaniasis caused by *Leishmania tropica* in North Jordan and other regions of the Middle East where this species is important.

The principal recommendations by the committee are as follows:

1. More statistical analysis of the data pertaining to the epidemiological and entomological data could be employed (for example, in the interpretation of the clustered distribution of cases).

2. The arguments for the zoonotic transmission of the disease, both in North Jordan and the Jordan valley foci, are suggestive but not yet convincing. The decision to focus on the identification of the reservoir species is commended. The committee feels that the role of hyraxes as a reservoir in North Jordan is doubtful, and that emphasis on other potential reservoirs nearer to human communities would be more productive.

3. Controlled studies should be conducted to better document the team's ability to use hamsters as a model for the study of *Leishmania tropica*, and these results should be published.

4. More efficient PCR methodologies need to be developed. The present system, which relies on differences in amplification to distinguish among two of the three *Leishmania* species under investigation, likely will not allow differentiation among these species when applied to samples obtained from the field.

5. Care should be exercised when employing a 'landscape epidemiology' approach. Distributional patterns of *Leishmania* species may be determined by both ecological and stochastic (i.e., historical) factors. While the North Jordan site does differ noticeably both in terms of its habitat type and the species of *Leishmania* it maintains (relative to other Jordanian sites), the committee feels that any inferences based upon this limited sample would be suspect. The Principal Investigator is encouraged to visit additional *Leishmania* foci, especially in Israel and Jordan, to increase the sample size upon which any 'landscape' generalizations are based. This also would be keeping with the spirit of the MERC program.

6. It is commendable that the investigators also are engaged in research on cystic hydatid disease. Their ability to produce protoscolices *in vitro* is impressive. However, it is curious that ELISAs here and in Israel are only 60% sensitive, while the Tunisians report 97% sensitivity. It is recommended that the MERC participants strive to arrive at a standard protocol for such parallel work.

Efforts towards establishing regional collaborations: The project has contributed to the design and implementation of the common MERC work plan. Researchers involved with the project have participated adequately in joint meetings sponsored by the MERC program. The sponsorship of the next Principal Investigator's meeting by the Jordanian Principal Investigator also is commendable. The committee feels that the geographic position of the Jordanian project, as well as the expertise of the research team there, gives this project the potential to play a pivotal role in the facilitation of collaboration, especially with the Lebanese and Israeli MERC projects.

Sub-Project 4: Leishmaniasis in Israel: Some Parasite Vector Reciprocal Effects
Principal Investigator: Yosef Schlein, Ph.D.Hebrew University of Jerusalem
Primary Reviewer: Philip Lawyer, Ph.D.

Background. *Leishmania* infections in sand flies are limited to the gut where contact with tissues, secretions and the medium of sand fly food influence their cycle of development. The parasites cope with and exploit their habitat through products that impair the function and damage the tissues of the gut. In previous studies, Dr. Schlein and colleagues found that *Leishmania* secrete chitinolytic enzymes. It was also observed that *L. major* parasites in the sand fly *Phlebotomus papatasi* caused damage to the insect tissues, enabling the development and transmission of parasitemias. Circumstantial evidence indicated that this damage was caused by chitinases of the parasites. Conversely, the vector's selection of natural diets is a variable determinant in the success of the parasites. The broad goal of this project is to contribute to the understanding of the parasite-insect vector interaction and its influence on the transmission of leishmaniasis.

The main objectives are:

1. to examine the role of parasite chitinases in development of infection in the sand fly gut and subsequent transmission by sand fly bite;

2. to assess the effects of plant feeding on the potential for *Leishmania* transmission by sand flies, identify the actual plant food sources of *Phlebotomus papatasi* in the Jordan Valley leishmaniasis focus, and assess the importance of the local flora on the rate of leishmaniasis transmission in the endemic region;

3. to identify the reservoir animals and sand fly vectors of *L. tropica* in the mountainous region near Jerusalem to the end that specific control measures against the disease may be employed.

Detailed Scientific Review:

Objective 1: To examine the role of parasite chitinases in development of infection in the sand fly gut and subsequent transmission by sand fly bite. The first stage of this work, purification of chitinase, involved culturing *L. major* parasites in Dulbecco's modified Eagle's medium (DMEM) to produce spent medium containing parasite-derived enzymes. This fraction was then passed through several steps, including gel filtration, hydrophobic chromatography, affinity chromatography and chromatofocusing, to produce purified chitinase enzyme. Obstacles in the purification process included low enzyme yield of cultured parasites and confounding chitinase activity present in fetal calf serum used as a standard additive to *Leishmania* culture media. The first obstacle was overcome when it was found that by using parasites harvested from infected animals, the high level of chitinase secretion was recovered. Several serum replacements were introduced to overcome the second problem. These replacements either had their own chitinase activity, or did not support parasite growth well. Nonetheless, protein precipitates from low-yield culture overlays were used for protein fractionation and a high degree of purification resulted. Dr. Samuel Martin at the Department of Entomology, Walter Reed Army Institute of Research, has applied for a patent on a serum/protein-free culture medium that works very well for culturing *Leishmania* parasites, and from which pure parasite secretions are easily isolated. This reviewer discussed Dr. Schlein's project with Dr. Martin, who expressed interest and willingness in letting

Dr. Schlein try some of his new medium for culturing parasites and increasing the yield of pure antigen.

The second stage of this objective included cloning the *L. major* chitinase gene. An *L. major* genomic DNA expression library was used, in which DNA fragments are inserted into a unique EcoR I site within the LacZ gene located 53 bp upstream of the ß-galactosidase translation termination codon. The inserted DNA is expressed, under the lac promoter, as a fusion protein including 100 amino acid residues of the ß-galactosidase N-terminus. The library was screened with 4-methylumbelliferyl chitotrioside. Two plaques which expressed ß-galactosidase-chitinase fusion protein were isolated, their DNA purified, mapped by restriction endonuclease cleavage and sequenced. To date, sequencing of the majority of both DNA strands of the *L. major* insert has been completed, with a few short regions to be re-sequenced. The positive plaques were grown in *E. coli* Y1089 cells and screened for chitinase activity. During the final year of this project this fragment will be used as a probe for the isolation of the *L. major* chitinase gene and following chitinase activity during the life cycle of the parasite in the sand fly. Such information will contribute significantly to an understanding of parasite-vector interactions that enable transmission and might be exploited in developing leishmaniasis control strategies. The researchers also plan to use specific DNA probes for detecting chitinase activity in wild-caught sand flies and for diagnostic purposes in detecting *Leishmania* parasites in samples from human infections.

Objective 2: To assess the effects of plant feeding on the potential for *Leishmania* transmission by sand flies, identify the actual plant food sources of *Phlebotomus papatasi* in the Jordan Valley leishmaniasis focus, and assess the importance of the local flora on the rate of leishmaniasis transmission in the endemic region. In earlier laboratory studies using the cold anthrone test to detect the presence of fructose in the sand fly gut, the PI demonstrated that *P. papatasi* feeds preferentially on certain plants,. This test proved inadequate for identification of plant food sources in the field since some plants contain levels of fructose too low to be detected. It is also unsuitable as a general indicator of plant feeding, since sand flies also feed on nectar and aphid honeydews that contain fructose. The investigators observed that *P. papatasi* ingests tissue particles including cellulosic shreds of cell walls during plant feeding. Based on this, they developed a staining method using calcofluor (Fluorescent Brightener 28), which is a specific stain for cellulose. Labelling the plant branches with calcofluor was problematic because the label would not penetrate the waxy cuticle of the leaves by simple immersion in the staining solution. Successful suffusion was accomplished by using alternating low pressure to release air from the intracellular spaces in the plant and replace it with staining solution. Labeled cellulose ingested in plant meals by sand flies was then detectable by fluorescence microscopy.

Two series of 100 unfed female *P. papatasi* were exposed to calcofluor-suffused branches for 24 hrs. The guts of the flies were then dissected and examined under the fluorescent microscope for the presence of labeled cellulose particles. A control series of flies was fed on untreated branches and their guts tested with anthrone. By calcofluor labelling, sand fly feeding was detected on at least five plant species that was not detected using the cold anthrone test. One plant, *Atriplex halimus*, is the main food source of the sand rat *Psammomas obesus*, the rodent reservoir of *L. major* causing human leishmaniasis in the Jordan Valley. This plant was not considered a food source for *P. papatasi* because flies exposed to it were negative by the anthrone test in earlier experiments. However, calcofluor staining revealed feeding by 30.5% of the flies. Based on these results, this plant, which is common near the breeding and resting sites of P. papatasi, is now considered a main plant-food source for *P. papatasi.*

Preliminary field experiments were conducted in which calcofluor-suffused branches of various plant species were presented together with sand fly traps near sand-rat burrows. The results showed that calcofluor-labeled branches can be used for specific identification of plants that are natural sources of food for sand flies. Future field experiments will be conducted to assess the importance of *A. halimus* and other plant species in the natural diet of *P. papatasi*. Such information will enable investigators to develop sand fly control strategies that specifically target the vector as it feeds on its preferred plant host. Since calcofluor labelling is not a procedure that can be done easily in the field, branches were suffused in the laboratory and carried to the field for experiments. It is not clear what damage this procedure does to the plant and whether it alters the attractiveness of the plant to the sand fly. It might be better if a method (perhaps PCR) could be devised to label or identify the cellulose of specific plants as they grow naturally in the sand fly's habitat.

In earlier studies Dr. Schlein and colleagues observed that feeding of *L. major*-infected *P. papatasi* on some of the plant species caused massive mortality in parasites. Because agglutination of parasites was observed in many of the infected flies, they assume that ingested plant lectins may be the major cause of this mortality. During the final year of the study, experiments are planned to test this hypothesis.

Objective 3: To identify the reservoir animals and sand fly vectors of *L. tropica* in the mountainous region near Jerusalem to the end that specific control measures against the disease may be employed. The team has been trapping mammals and sand flies in and around the village of Kfar Adumim in an effort to incriminate both the reservoir(s) and vector(s) of recently reported human cases of *Leishmania tropica*. Using 150 baited animal traps during 15 trapping nights, 2 hyrax, one shrew and 39 murid rodents, including Acomys, Rattus and Mus were collected. The animals were necropsied and smears and cultures taken from body tissues and fluids. All smears and cultures were negative. However, ELISA tests of animal sera produced two positive *Mus musculus*. The PI pointed out that whereas this finding may be indicative of a transitory infection with *Leishmania*, the question of cross-reactivity with other rodent parasites must and will be addressed.

Over 500 sand flies were collected on sticky traps in and around the village of Kfar Adumim, of which 152 females were dissected and examined for parasites. All dissected sand flies were negative for *Leishmania*. Because this was a relatively small sampling of flies to examine, it is not surprising that no infections were observed. Often thousands of flies must be examined before one infected fly is found. No human- or animal baited collections were conducted. At least 20% of bloodfed females had nucleated red cells in the bloodmeal, indicating that they had fed on avian or saurian blood. The males and remains of the females were mounted for identification; all were identified as *P. papatasi*. This sand fly species has been shown by others to be incapable of transmitting any *Leishmanias* other than *L. major*. Therefore, it is not considered a potential vector in this case and the search continues for other species such as *P. sergenti*, an incriminated vector of *L. tropica*.

Previous reports of positive smears of *Leishmania* from *Rattus rattus* at Salfit, in the central highlands, suggested that this animal may be a reservoir host at Kfar Adumim, which has a similar biotope. To help resolve this question, the research team examined three biological criteria: 1) the exponential growth of promastigotes of *L. tropica* in culture media supplemented with 10% blood from different mammalian sources: 2) the infectivity of these promastigotes to cultures of peritoneal macrophages from rats; and 3) the infectivity of the isolate to different anatomical sites in the rat. The results of these experiments showed that the isolate grows better in other

mammalian blood than in *Rattus rattus* blood. Inoculation of the local strain of *L. tropica* into wild and inbred *Rattus rattus*, did not produce external lesions, but did result in positive cultures from spleen, footpads, ears, nose, lymph glands and spleen. Serum antibody levels were positive only for some rats. Hamsters injected with the local strain of *L. tropica* had swollen footpads and positive smears and cultures at three weeks post injection, whereas those injected with an anthroponotic strain from Turkey were apparently negative after 2 months. SCID mice showed no sign of lesions. Macrophages from rats were more easily infected than those from other hosts but only 25% became infected. In further laboratory experiments using wild-caught *Rattus rattus*, adult rats showed various responses to *L. tropica* infections, from transitory infections at the site of injection to visceral metastasis. They concluded that it is possible that some rats may act as a reservoir in the *L. tropica* focus under study. Efforts continue to confirm or refute this in the field.

Summary: The Israeli team, under the direction of Dr. Schlein, has accomplished major portions of all three objectives through rather innovative, original and productive research, and it appears likely they will accomplish the remaining portions of all three by the end of the third year. In the process they have developed new skills and techniques that will not only benefit their future studies but those of other MERC participants. Three publications in international scientific journals and two oral and one poster presentation at an international scientific conference have resulted from this research so far.

The most difficult stages of the first objective, purification of *L. major* parasite chitinase and cloning and sequencing the corresponding gene have essentially been accomplished. Obstacles encountered during parasite culturing were overcome sufficiently to enable purification of small quantities of parasite chitinase. Cloning *L. major* genomic DNA in the *E. coli* LacZ gene produced two plaques which expressed ß-galactosidase-chitinase fusion protein. DNA from these plaques was purified, mapped and the majority of both DNA strands sequenced. The positive plaques were grown in E. coli Y109 cells and screened for chitinase activity. During the final year of the project, refinements will be made in the culture medium to increase the yield of purified parasite-chitinase. Portions of the *L. major* chitinase gene will be resequenced, and the gene will be used as a probe to follow chitinase activity during the life cycle of the parasite in the sand fly.

As pertains to the second objective, techniques were developed for labelling branches of various plant species with calcofluor and exposing sand flies to them to evaluate their feeding preference. One plant that is common in the sand fly habitat, *Atriplex halimus*, had been discounted as a possible food source for *P. papatasi* based on earlier experiments using the cold anthrone test. Calcofluor staining revealed feeding by 30.5% of the flies. This plant is now considered a main plant-food source for *P. papatasi*. During the final year of the study, the researchers plan to use this new test in field experiments to identify specific plants that are natural sources of food for sand flies. They will also test the hypothesis that lectins of certain natural plant food sources cause mortality of *L. major* in *P. papatasi* .

With regard to the third objective, efforts to identify the reservoir animals and sand fly vectors of *L. tropica* in the mountainous region near Jerusalem through trapping and parasitologic examination have been unsuccessful. Whereas field collections have not led to incrimination of either reservoir or vector, the results of the laboratory experiments showed that macrophages from rats were more easily infected than those from other hosts, and that wild-caught *Rattus rattus* adults showed various responses to *L. tropica* infections ranging from transitory infections at the site of injection to visceral metastasis. The researchers concluded that it is possible some rats may act as reservoirs in the *L. tropica* focus under study. Renewed efforts to collect and examine mammals and sand flies from the area will be necessary during the final year to accomplish this

objective. This project was not mentioned in the latest semi-annual work plan, but the research team plans to continue the search for reservoir(s) and vector(s) in Kfar Adumim.

Recommendations from the committee are as follows:

1. In cultures of *L. major*, try Martin's serum/protein-free medium to increase the yield of parasite-derived chitinase proteins.

2. In reservoir infectivity studies in the laboratory, allow at least six months for *L. tropica* lesions or visceral symptoms to appear on animal models.

3. Investigate the possibility of developing PCR techniques for differentiating ingested celluloses of various plants in the sand fly gut. This would overcome the artificiality of vacuum-aided suffusion of calcofluor into plant branches.

4. In addition to sticky (castor oil) traps for collecting sand flies at Kfar Adumim, conduct resting site collections with mouth aspirators in early morning, and all-night human- and animal-baited collections to collect man biters, thus increasing the diversity of species collected and the likelihood of finding a vector.

Efforts Toward establishing regional collaborations: Due to the nature of the basic research conducted thus far, this project has contributed marginally to the design and implementation of the common MERC plan. Other than participation in semi-annual PI meetings, formal cooperation with Arab counterparts has been rather limited. A two-week visit by Dr. Mulkiye Kasap from the University of Cukurova in Adana, Turkey, was co-hosted by Drs. Jaffe and Schlein in September 1995. Dr. Kasap was trained by Dr. Schlein in techniques for collecting and identifying sand flies. The team's chief concern has been with the science of the projects rather than with developing regional cooperation. However, now that the mechanisms and procedures for achieving the project objectives have been worked out, the PI and colleagues should be in a better position to establish research partnerships and cooperative relationships with other MERC participants. The expertise of Dr. Joseph Shlomai's laboratory is in molecular biology and enzymology of trypanosomatids. Areas where they could and should develop cooperative projects with Arab partners are: 1) in providing specific DNA probes for detecting chitinase activity in sand flies, for diagnostic purposes in samples from human infections and for the detection Leishmania parasites in infected flies from the field; 2) in providing training to cooperators in obtaining specific DNA probes for diagnostic purposes, by PCR and other methodologies routinely in use in their laboratory; 3) in training and assisting cooperators in preparing appropriate cDNA and genomic DNA libraries from the parasite cells under study and their screening for particular genes under study and; 4) in providing assistance and training in protein purification, cloning and characterization; DNA enzymology and topology.

Because cutaneous leishmaniasis caused by *L. major* occurs with hyperendemicity in virtually all of the MERC countries, the Israeli team should endeavor to collaborate with MERC Network partners, particularly the Jordanian team, in parallel or complementary studies directed toward a common strategy for control. They could also exchange isolates, share data and procedures, and exchange training in specific methods they have developed in the course of their experiments. It is suggested that a specific collaboration be initiated with the Jordanian research group to study similarities and differences in *L. major* transmission on both sides of the Jordan Valley. The phenomenon of *L. tropica* appearing as an emergent zoonosis in hill-top/ridge-top settlements is also a problem of mutual interest in at least four of the five countries visited (Israel, Jordan,

Tunisia and Morocco) that warrants regional scrutiny. The same holds true for visceral and cutaneous *L. infantum* in these countries. Such cooperation could produce the first truly regional perspective of patterns of leishmaniasis transmission in the Mediterranean basin. That the Jordanians have found hamsters to be a good model for *L. tropica* may be another point of mutual interest with the Israelis and a good basis for collaboration. Dr. Shaden Kamhawi, the PI of the Jordanian project, has been invited to visit Hebrew University to review the Israeli projects and perhaps initiate collaborations.

Sub-Project 5: Ecology, Epidemiology and Study of Risk Factors of Leishmaniasis in Tunisia
Principal Investigator: K. Dellagi, M.D.Institut Pasteur, Tunis, Tunisia
Primary Reviewer: Franklin Neva, M.D.

Background: The original objectives were:

1. Comparative evaluation of classical and modern tools for diagnosis and eco-epidemiological investigation of leishmaniasis.

   - Use of molecular probes for identifying *L. infantum* from clinical specimens.

   - Development of ELISA tests with defined *L. infantum* antigens.

2. Evaluation of risk factors for development of visceral and cutaneous leishmaniasis

   - By study of cell-mediated immune responses to leishmanial antigens in patients with visceral leishmaniasis and in asymptomatic children, and by an immunohistochemical analysis of cellular infiltrate into skin lesions with particular reference to expression of mRNA for cytokines.

   - By analysis of genetic factors that may influence susceptibility or resistance to disease by HLA typing.

   - By analysis of familial distribution of visceral leishmaniasis in the governorate of Kairouan and the familial relationship to dogs, using geographic mapping techniques.

3. Identification of vectors and reservoir hosts involved in transmission of leishmaniasis in Tunisia, especially

   - Wild animal reservoirs of *L. infantum*

   - Vector and reservoir hosts of *L. killicki* in South Eastern Tunisia.

Detailed Scientific Review:

Objective 1: Comparative evaluation of classical and modern tools for diagnosis and eco-epidemiological investigation of leishmaniasis. Progress on the development of DNA-based methods to detect *L. infantum* has been good. The person working on this part of the project, Dr. Ikram Guizani, presented her most recent work on identification of leishmanial isolates and of clinical specimens using molecular probes prepared from kinetoplast DNA. She had prepared two probes that were specific for *L. infantum* and could detect between ten and one hundred nanograms of total parasite DNA. One of the probes was more sensitive than the other, with

sensitivity down to nearly ten nanograms of DNA. Both probes were quite specific in that they failed to cross react with as much as one microgram of many strains of *L major* and *L. tropica.*

These kinetoplast DNA probes were also employed in preliminary studies to detect parasites in bone marrow aspirates from patients with visceral leishmaniasis, and lymph node and splenic aspirates from dogs that had been sacrificed with canine leishmaniasis. In these situations sensitivity of the DNA probes was not as good as when parasite cultures were used. Furthermore, the sensitivity of the DNA probes when used on clinical samples suggests that the assay as it is now designed will be of marginal usefulness for the identification of clinical samples, and for the identification of parasites in infected sand flies. The investigators are encouraged to attempt to adapt their DNA probes for use with PCR to improve the sensitivity of their assay. Furthermore, the poor sensitivity of the DNA probes when used on cultured parasites may reflect deficiencies in their classical culture techniques, apart from suggesting poor sensitivity of the DNA assay itself.

Dr. Guizani clearly has the background and training to do the type of molecular biology proposed for this part of the project. But it is disappointing that some of the classical parasitology does not seem to be as effective as it should be. An example of this is the inability to culture leishmania from clinical specimens. Without such a reliable bench mark or 'gold standard' , how can the results using sophisticated molecular methods be evaluated? However, the results on using two different kinetoplast minicircle probes from *L. infantum* for identification and differentiation from other species of *Leishmania* appear to be promising.

Furthermore, it is apparent that the other projects of the leishmaniasis module are not currently using molecular probes for identification of *Leishmania* species. Thus, the Tunis group could provide a useful reference service for the MERC program. Dr. Guizani has an impressive list of publications and presentations at congresses or meetings, so her research productivity has been more than adequate.

The second major goal of this objective was to attempt to develop an ELISA assay based upon defined parasite antigens, which would prove to be superior to one based on crude parasite preparations. Although the overall goal was to evaluate such defined ELISA assays, the first Progress Report included results of a serosurvey of dogs from the Medjez El Bab area 60 km to the west of Tunis. A similar survey had also been carried out in 1990-91. A crude soluble *L. infantum* antigen was used in the ELISA test. Surprisingly, the results of these studies were not described, either in the first progress report or Dr. Dellagi's oral presentation.

In order to develop a more specific ELISA assay, several different partially purified or recombinant antigens of *L. infantum* were obtained and tested for reactivity against sera from visceral leishmaniasis patients. These included:

- Gp42(recombinant) from Dr. Handmann of Australia
- Gp63(recombinant) from Institut Pasteur in Paris
- purified P14
- purified P16
- purified P24
- purified P32
- purified P94
- a160

A great deal of work was done in attempting to further purify some of the antigens, especially P32. In addition, sera were tested with different combinations or mixtures of antigens in efforts to improve sensitivity and specificity of tests. However, the rationale of some of these experiments, and their relevance to the overall goal of the project were not clear. In particular, given the fact that none of the purified antigens appeared to result in an assay that significantly improved on the crude antigen ELISA leads one to question why such a large amount of effort was directed towards following up this avenue of research. A more practical strategy of simply selecting a crude antigen and defining the best conditions for its use, even if it has some disadvantages, would have perhaps served the overall objectives of the project better.

Objective 2: Evaluation of risk factors for development of visceral and cutaneous leishmaniasis. This research objective consisted of several components, which are emphasized to varying degrees in different six month Work Plans and the three Progress Reports. The emphasis given to each component of this general research objective in the oral presentations was not always consistent with the emphasis in the Work Plans and Progress Reports. For example, little if anything was said about investigation of cell-mediated responses to soluble and membrane antigens of *Leishmania* in the work plans or progress reports. Yet Dr. Dellagi spent a considerable amount of time in discussing these results in the oral presentation. This deviation from Work Plan concerned the finding of a high prevalence of cell-mediated immune responses by peripheral blood cells (i.e. stimulation indices or blast transformation) to leishmanial membrane antigens. These occurred in 50 to 60 percent of individuals tested, whereas only 30% or so of the same individuals responded to soluble leishmanial antigens. Even cells from cord blood responded to the membrane antigen preparations, suggesting a mitogenic or non-specific effect of the membrane antigen. However, the proliferative response could be inhibited by anti-Class II MHC antibodies, suggesting that Class II antigen presentation was required for the proliferative response. Dr. Dellagi concluded that leishmanial membrane antigen was behaving like a super antigen. One questionable point in this story was that a Swedish immunologist had not been able to reproduce these results. At any rate, this investigation did not fit into the overall research objectives of this project as originally stated.

Although it does not easily fit into the category of a risk factor for development of disease, the study done by Dr. Hechmi Louzir on cytokine expression of inflammatory cells into lesions of cutaneous leishmaniasis was well-planned and carried out. It also served to define the natural history of cutaneous leishmaniasis in a region 400 km south of Tunis (near Gafsa), because it included a comparison of intralesional treatment with antimony vs. no treatment in a comparable group of patients. The study, done in early 1995, involved a total of 112 patients with lesions present for an average of 56 days. Biopsies of lesions were quick frozen in liquid nitrogen. Half of the samples were reserved for immunohistochemical analysis and half for RNA extraction. The RNA extracted from the lesions was then analyzed for the presence of cytokine mRNA by semi-quantitative RT-PCR. Dr. Louzir spent two months in Dr. Peter Melby's lab in San Antonio, Texas doing the cytokine assays. A statistical analysis of the presence and amount of each cytokine correlated with other cytokines was presented in the third progress report, and orally at the site visit. Although there is still further analysis of results to be done, it does not appear that any unusual patterns of cytokine response were observed in the lesions. However, very few studies such as this have been done, so this investigation provides a base line for other future work. One of the most interesting outcomes of the study was the finding that 90 percent of the lesions had cured spontaneously without treatment by day 105. This type of information is very useful in defining the nature of cutaneous leishmaniasis in this area. It would also be worthwhile to obtain similar information on the time course of the disease without treatment in the other MERC countries.

The original research proposal by the Tunisian group indicated, in considerable detail, plans to investigate possible genetic control of susceptibility (or resistance) to human visceral leishmaniasis. This was to be done by analysis of MHC genetic polymorphism in patients, in asymptomatic but infected, and in non-infected cases. This was to be done in two different areas, the governorate of Kairouan (south of Tunisia) and in the Medjez El Bab area (60 km west of Tunis.). In addition, HLA Class I and II typing of patients in the cutaneous leishmaniasis study in the south of Tunisia was proposed in the second progress report. Although no information on the HLA typing results was included in the progress reports, an oral presentation was given by one of the Institute staff during the site visit. Apparently some of the typing work has been started, but results are still very preliminary. However, the committee had serious doubts that the procedures being used by the investigators will yield meaningful results. HLA genetic analysis is a sophisticated business requiring large numbers of subjects, careful delineation of family trees and relationships, and suitable controls. It appears that such a careful sampling strategy is not part of the current study. For example, some of the numbers of cases to be studied discussed included 46 unrelated visceral cases, 50 individuals without disease but from an endemic area, and 150 country-wide controls. This sample is clearly insufficient to allow one to draw any statistically significant conclusions from such a study. Furthermore, one of the critical groups that should be included in an analysis such as this is a group that has had inapparent infection but no obvious disease. For leishmaniasis, such individuals could be identified by use of the leishmanin skin test. The investigators have apparently not considered including such a group in their study.

Objective 3. Identification of vectors and reservoir hosts involved in transmission of leishmaniasis in Tunisia. Of the three major objectives in the original proposal, this was the least developed at the time of the site visit. Although this topic was addressed in the original Technical Research Proposal, it was not mentioned further in the individual work plans, and in none of the progress reports. In fact, it can be observed that the Tunisian studies have focused mainly upon the parasite and the immune response of infected individuals to the parasite. The epidemiological studies have lagged, and studies of the animal reservoirs (other than the dog) are not under study at all. Similarly, no information was provided in either the progress report or in the oral presentations describing any data obtained regarding studies on the vector of the parasite.

In regard to the epidemiologic study of visceral leishmaniasis in the Kairouan area, the committee had some concerns as to the timing of different components of the study. The index cases of visceral disease which form the basis for the study occurred some time ago. The survey being done now will focus upon family contact with dogs. But will it concern present contact with dogs, or past contact with dogs when the cases actually occurred.? It is not clear that the information obtained by recall as to dog contact several years ago will be reliable.

Efforts towards establishing regional collaborations: Drs. Dellagi and Guizani attended the Principal Investigators' meetings in Bethesda (4/94 and 4/95), Izmir, Turkey (10/94) and San Antonio, Texas (11/95). DNA preparations from 19 strains of leishmanial parasites from Lebanon were sent to the Tunis lab and checked as to species by Dr. Guizani. Similarly, serum samples from Dr. El-On of Israel and 18 serum samples from Dr. Salti of Lebanon were sent to the Pasteur Institute of Tunis for testing as part of the project. The Tunisian laboratory has hosted visitors from Jordan, Morocco and Lebanon for consultations about techniques used in the MERC protocols. An epidemiology workshop for MERC program members is scheduled to be held in Tunis in May, 1996. Therefore, there has been support of the common work plan, and more interaction with the other MERC labs is planned.

Sub-Project 6: Hydatid Disease in Israel
Principal Investigator: Dr. Joseph El-On, Ben Gurion University, Beer Sheva, Israel
Primary Reviewer: Dr. Thomas R. Unnasch

Background: Historically, hydatid disease has been an important public health problem in Israel, as it is throughout much of the Middle Eastern Region. However, a series of rabies elimination campaigns, coupled with a ban on animal importation reduced the prevalence of hydatid disease in the state of Israel. In contrast to this overall trend, a significant outbreak of hydatid disease was discovered recently in the northern Israeli village of Yirka. Epidemiological studies of the population of Yirka revealed that approximately 1.6% of the population were seropositive to *Echinococcus granulosus* antigens. This suggested that *E. granulosus* infection might represent an emerging public health problem in the Galilee area of Israel. The overall goal of this proposal was to further investigate this potentially important emerging health problem in the Galilee, and to develop a strategy to control the infection if a problem was indeed detected. The specific aims of the proposal were:

1. To determine the prevalence of *E. granulosus* infection in humans and dogs in another study site geographically separated from the previously identified focus of Yirka.

2. To evaluate and refine the current diagnostic methods for *E. granulosus* infection.

3. To assess parasite specific cellular and humoral immune responses to *E. granulosus*, and to follow any changes in these responses as a result of surgical or drug treatment.

Detailed Scientific Review: To address the specific aims of the proposal, the investigators identified a study site which was distinct from the previously identified focus of Yirka. The site selected was the village of Tamra, an Arab community located approximately 10 km from Yirka. The investigators initiated a public education program, and obtained the cooperation of the local authorities. As a result of these efforts, the investigators were able to conduct a complete census of the human and dog populations of Tamra. The investigators also collected approximately 9000 blood samples, to measure seroprevalence in the population. The investigators have clearly obtained a high level of cooperation from the community, a key prerequisite for carrying out the type of epidemiological study that was envisioned in the original proposal. Furthermore, the majority of blood samples collected by the group have been analyzed, and the results reveal that the seroprevalence rate in the study village is approximately 2.2%. These results demonstrated that *E. granulosus* infection is not restricted to Yirka, and that this infection may represent an emerging health problem in Northern Israel.

Dr. El-On and his colleagues have also undertaken a series of studies to evaluate and refine the currently available diagnostic assays for *E. granulosus*. Initial investigations demonstrated that the crude hydatid cyst fluid (HCF) antigen preparation used in the standard ELISA assay was heavily contaminated with host proteins. The investigators developed a purification scheme for the parasite antigens, and used this purified preparation to refine the serological assays for detection of the parasite infection. These results suggested that two previously identified antigens, antigen 5 and antigen B, were the most effective for the detection of *E. granulosus* infection. Assays based upon these purified antigen preparations appeared to be species specific. Unfortunately, further studies suggested that serological assays based upon the purified antigen preparations were rather insensitive. It is possible that further refinements in this assay, such as adjusting the cutoff for the assay, or using the ELISA in combination with other immunological assays such as IFA may result in a test that is more sensitive than the current ELISA. The

investigators are encouraged to continue to attempt to refine these assays, to further increase their sensitivity. Furthermore, the Tunisian members of the MERC project have conducted parallel investigations of the HCF ELISA assay, and have data which suggests that it is much more sensitive than the results of Dr. El-On suggest. Dr. El-On and his colleagues are encouraged to collaborate with the Tunisian group to develop a standardized ELISA assay protocol that may be applied by all members of the MERC program working on hydatid disease.

Dr. El-On and his colleagues have also begun a series of studies investigating the cellular immune responses in individuals infected with *E. granulosus*. These studies have suggested that cellular immune responses to parasite antigens may be detected in the large majority of individuals infected with *E. granulosus*. Given the character of the infection, this is not surprising. Furthermore, the approach used by Dr. El-On to characterize these cellular responses is rather crude. One rationale given by Dr. El-On for undertaking these studies was that such cellular immune responses might be a useful adjunct to detect infections missed by the ELISA assay. However, the cellular assays utilized by the investigators are too complex to be used in routine epidemiological studies. Dr. El-On is encouraged to use more sophisticated methods to analyze the cellular immune response in infected individuals, and to abandon the rationale of developing cellular assays as an alternative diagnostic technique.

Dr. El-On has also carried out a study of the changes in immune responses following either surgical or chemotherapeutic treatment of confirmed cases. These results have suggested that the immune response to parasite antigens persists for years following treatment. However, it is not clear if this is due to a particularly long lived immune response to parasite antigens, or to the possibility that a sub-clinical infection remains in individuals following treatment. Further experiments, including longitudinal follow-up of treated individuals, will be necessary to differentiate these possibilities. In addition, a more sophisticated approach to the study of the character of the cellular immune response may demonstrate, that while the overall response persists over time, the type of response may be modified following treatment. Nonetheless, it is clear from these results that simple immune correlates will not be useful in measuring the efficacy of treatment in *E. granulosus* infection.

Summary The investigators of this project have made substantial progress towards answering the questions posed in the original proposal. They have demonstrated that *E. granulosus* represents an emerging health problem in Northern Israel, and have mechanisms in place to measure the effect of various control measures on the prevalence and incidence of infection. However, the laboratory studies, and in particular those involving the cellular immune responses induced by *E. granulosus* infection, have proven less fruitful than the epidemiological studies. It is suggested that these studies be re-evaluated and redirected in light of the results obtained to date.

No publications have resulted from this project at the current time. However, the authors have presented their results at international meetings, and plan to publish their data on the coproantigen study in the near future. Thus, although the productivity as measured by publications is not great, this should be re-evaluated at the end on the project period.

Efforts towards establishing regional collaborations: Dr. El-On and his group have participated in the multicenter evaluation of the coproantigen assay carried out in conjunction with scientists in Jordan and Tunisia. They have also collaborated with the Tunisian group in identifying a common work plan for carrying out the epidemiological studies of hydatid disease to be carried out by the MERC program. Dr. El-On has attended all of the MERC joint meetings held to date.

Sub-Project 7: Community Based Study of the Transmission Dynamics of Hydatidosis
Principal Investigator: Riadh Ben-Ismail, M.D. Institut Pasteur, Tunis, Tunisia
Primary Reviewer: Thomas R. Unnasch, Ph.D.

Background Hydatidosis represents one of the major health problems of Tunisia, with 1-5% of the rural population being infected with the causative agent of the disease, *Echinococcus granulosus*. The major treatment for the disease is surgical intervention, and Tunisia performs approximately 1200 surgical excisions of cysts per year. This represents a major investment of health care resources by Tunisia, making hydatidosis an important economic drain on the health care system of the country. The development of an effective control program for hydatidosis would therefore have a major impact on the health of rural Tunisians, as well as serving to free up scarce health care resources of the country for other purposes. The overall goal of this project is to perform a detailed study of the ecology and epidemiology of the disease. The information gained from this study will be used to design a targeted control program. To reach this overall goal the specific aims of the project are:

1. To study the prevalence and distribution of *E. granulosus* infection in humans and dogs, which serve as the definitive host of the infection

2. To estimate, through prospective studies, the stability of the disease foci in both humans and dogs

3. To identify the major routes for dispersal of the eggs of the parasite, and to investigate various human food sources for contamination by parasite eggs.

Detailed Scientific Review:

To accomplish the specific aims of the proposed work, it was first necessary to obtain an accurate census and detailed map of the study area, and to develop sensitive and specific diagnostic methods for detecting the infection in both the human and dog populations. The major research effort over the first two years of the project has been directed towards fulfilling these two prerequisites. The investigators have made substantial progress in fulfilling the first prerequisite. The cooperation of the local authorities has been obtained, which has allowed the investigators to conduct a careful census of the entire dog and human populations in the study area. Detailed maps of the study site have been produced, and these have been entered into the geographic information system (GIS) established by the investigators. The investigators have thus obtained all of the necessary census and topographical information necessary to complete the project as originally envisioned.

Apart from the field work described above, the major emphasis of the investigators during the past two years has been on fulfilling the second project prerequisite, that of developing highly sensitive and specific diagnostic methods for detecting the infection in both the human and dog populations. The investigators have conducted studies validating the use of the crude hydatid cyst fluid (HCF) ELISA assay for the detection of infection in humans. These studies have suggested that the HCF ELISA was greater than 95% sensitive and 95% specific for the detection of infection in humans. This degree of sensitivity and specificity is quite high, especially in view of the fact that the same assay has been found to be approximately 60% sensitive in similar studies conducted by the MERC groups in Israel and Jordan. Furthermore, the investigators have invested a large degree of effort in attempting to refine the HCF ELISA, investigating the use of various refined antigen preparations in the ELISA, as well as looking at the use of antigen preparations derived

from other life cycle stages. As the original HCF ELISA appears to be highly sensitive and specific in the hands of these investigators, the degree of overall effort that has been invested in this goal appears to be excessive, given that refinement of the assay is not one of the major goals of the project.

The investigators have also conducted a large scale study to determined the sensitivity and specificity of the arecoline purge assay for the detection of *E. granulosus* infections in dogs. The outcome of these investigations has suggested that, while the specificity of this assay is 100%, the sensitivity is only 70%. Furthermore, the arecoline purge procedure is unsuccessful in 14% of the animals tested, meaning that 14% of the animals could not be evaluated using this procedure.

The PI feels that neither of the above assays are sufficiently sensitive and specific to meet the requirements of the study that he plans to conduct. This is due to the fact that he feels that in order to conduct the spatial distribution studies proposed under Specific Aims 1 and 2, it will be necessary to reliably determine the infection status of individuals. In this case, the diagnostic tests he plans to employ must exhibit high positive and negative predictive values over a wide range of prevalences. Thus, Dr. Ben Ismail feels that it will be necessary to further refine the diagnostic techniques before addressing the major objectives of the study.

Summary The investigators have spent the past two years attempting to collect topological and census data, and in attempting to refine the diagnostic methods needed to carry out the major specific aims of the project. Thus, the major goals of the project remain to be addressed. It is hoped that in the final year of the project the investigators will make it a priority to apply the knowledge obtained during the first two years to address the major aims of the project. The review committee recognized that the assays available to the PI are imperfect in the sense that they may lack the versatility to reliably diagnose individual cases under different rates of infection prevalence. However no data have been presented to suggest a dramatically better assay will soon be available. The committee feels that the current assays are sufficiently reliable to allow the PI to begin to collect prevalence data that will allow him to begin to test the major hypotheses of the proposal. This is that the prevalence of hydatid disease is highly focal, and most cases will be concentrated in areas where improper slaughtering practices are maintained. The committee thus recommends that Dr. Ben Ismail utilize the data and tools he currently has on hand to begin to address the major goals of the original proposal.

A second recommendation of the committee concerns the HCF ELISA assay to diagnose human infections. In Dr. Ben-Ismail's hands, this assay appears to exhibit greater sensitivity and specificity than is found by the others. The committee recommends that Dr. Ben-Ismail collaborate with the other members of the MERC program to develop a uniform protocol for this assay, whose reliability will therefore be standardized throughout the MERC laboratories.

At the time of this review, no publications have resulted from this project. However, some of the collaborative studies, in particular the evaluation of the coproantigen assay, appear to be at a point where they may be ready for publication. Productivity should thus be re-evaluated at the end of the project period.

Efforts towards establishing regional collaborations:

This project has adhered to the common work plan put forth by the MERC committee. The Tunisian group has provided antigens for testing of the HCF ELISA assay to the Israeli group, and participated in the joint evaluation of the coproantigen assay in conjunction with the Israeli and Jordanian groups. The group will also sponsor the MERC GIS workshop involving all of the MERC

groups later this year. The PI has also participated in the joint meetings held by the MERC program during the past two years.

Sub- Project 8: Profile of Leishmaniasis in Lebanon (Epidemiology of Leishmaniasis in Lebanon and Characterization of Local Strains Using Immunological and Molecular Biology Methods)
Principal Investigator: Nuha Nuwayri-Salti, Ph.D. American University, Beirut
Primary Reviewer: Philip Lawyer, Ph.D.

Background: Cutaneous leishmaniasis is a well-defined clinical entity in Lebanon, but because of the recent war and resulting loss of infrastructure, case reporting is poor at best and little is known about the prevalence and distribution of the disease. Both *Leishmania major* and *L. tropica* had been implicated as causative agents of this disease in Lebanon, based on clinical manifestations, but specific characterization of isolates was not done. Just prior to commencing this study, the PI and one of her co-investigators (EB) assembled a group of 20 Lebanese isolates, 15 of which were obtained from patients presenting with cutaneous lesions at the American University of Beirut Medical Center between 1983 and 1992. These isolates were subsequently characterized by Dr. Richard Kreutzer, Department of Biology, Youngstown State University, Youngstown, Ohio. The investigators were surprised to discover that most of the isolates were not *L. major* or *L. tropica* as had been expected, but were mostly *L. donovani sensu lato*. Only 3 isolates were *L. major* and none were *L. tropica*. These findings underscored the need for further study, which eventually led to this MERC project.

The overall goal is to conduct a detailed assessment of the epidemiology and ecology of both cutaneous and visceral leishmaniasis in Lebanon. Information derived from the study will be used to design control strategies to improve the health of the Lebanese people. Specific objectives toward reaching this goal are:

1. To conduct a national human epidemiology survey .
   - contact/inform health-care providers
   - conduct public education (propaganda) campaign
   - confirm diagnosis of CL or VL cases by biochemical/molecular means.

2 To identify potential vector sand flies associated spatially and temporally with leishmaniasis cases.
   - trap and collect sand flies
   - identify sand flies
   - dissect and examine sand flies for *Leishmania spp.*

3. To identify potential reservoirs associated spatially and temporally with leishmaniasis cases.
   - trap potential reservoir mammals associated with case sites.
   - collect tissue samples and analyze for *Leishmania spp*

Detailed Scientific Review:[1]

Objective 1. To conduct a national human epidemiology survey: Because there was a strong sense that the disease was under reported, a concerted effort was made to inform health professionals of the disease and its manifestations. Initial contacts were made with officials from the Ministry of Health, Ministry of Education, private primary and tertiary health delivery institutions, and with leaders of religious organizations. These contacts were selected based on passive detection of cases. A "propaganda" campaign followed during which public education materials were refined and disseminated via radio, television and the press. As a direct result of the "propaganda" campaign, 18 patients were referred from different regions of the country. Villages from which suspected leishmaniasis cases were reported, were then targeted for house-to-house surveys. Five rural Akkar villages, 2 urban communities of Tripoli and 9 sectors of Beirut were canvased using a general demographic questionnaire to record number of occupants per household, etc. and a more detailed case-history questionnaire to record the particulars of suspected cases. The estimated total population surveyed at the time of this review was 50,000. In one subset of 3,390 households and a total of 21,566 inhabitants, 53 individuals were found with lesions consistent with CL; 72% of these were from one village. Two cases of VL were reported, one from Tripoli and one from Beirut. Limited skin testing with an Iranian antigen was conducted in villages, mostly in school children. So far only 3 positive skin tests have been observed. House to house surveys continue in Beirut and in rural villages where suspected cases have been reported. Whereas the information derived from these surveys is useful epidemiologically, it does not maximize case finding. Rather than looking for suspicious lesions the team should focus on mass screening using suitable skin tests. This would alert them to inapparent VL as well as CL and paint a better picture of the overall importance of the disease in the population. It was suggested by a member of the evaluation team (FN) that a local antigen might be more sensitive.

Efforts have been made to establish a capability for isolating, culturing and characterizing parasites using electrophoresis, polyclonal and monoclonal antibodies, excreted factor and DNA typing. Progress was slowed due to delays in receiving equipment and supplies, and only the electrophoresis system is ready and operating at present. A retrospective study of patients' records dating from 1979 through 1994 showed good success in culturing parasites from whole blood and from the buffy coat. This seems unusual for classical cutaneous leishmaniasis, since in such cases there is usually a paucity of circulating parasites. Since most of these cutaneous cases are suspected to be due to *L. infantum* (a typically visceral parasite), the finding of parasites in the buffy coat may not be too surprising. Lesion aspirates from new cases are routinely cultured on NNN medium with 30% rabbit blood and an overlay of fetal bovine serum and antibiotics. Isolation success has been about 35%. The PI says that the low success rate is not due to contamination, but due to failure of the parasites to grow in the culture medium. Because they lacked the restriction enzymes and PCR facilities, extracted samples of DNA from cultured parasites were sent for DNA typing to Dr. Ikram Guizani, of the Pasteur Institute, Tunis. Results of these typings had not been received. Rather than trying to develop in house capability for all characterization diagnostic technologies, the team should focus on electrophoresis capabilities and depend on other MERC laboratories to confirm the diagnosis using their specialty techniques (excreted factor and monoclonals, Israel; PCR, Tunisia; etc.)

Objective 2. To identify potential vector sand flies associated spatially and temporally with leishmaniasis cases. At the outset of the study, very little information was available on the potential vectors of leishmaniasis in Lebanon. Collection of phlebotomine sand flies was initiated with a visit from Dr. Nicolle Leger, Professor of Parasitology, Faculty of Pharmacology, University of Rheims, France. The purpose of the visit was to train Lebanese scientists in sand fly habits

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[1] The reviewers note that " Clearly our inability to visit the laboratories and the field sites, and [having] simply to hear about things from a distance by the Principal Investigator, was a tremendous disadvantage . . . some of the negative evaluation may be partly related to not being able to appreciate the conditions under which the [scientists] have to work in Lebanon."

biology and ecology, and in methods for collecting, mounting and identifying them. Initial collections were made in Merkabta village, Tripoli area, where a few cases of CL had been reported, and in a suburb of Beirut. During her visit in August 1994, Dr. Leger collected 7 species of sand fly, 5 of which are new records for Lebanon. The current team entomologist, Dr. Effat Abou-Fakhr, joined the project in August 1994, and began collecting with oiled papers and light traps in 4 villages of northern Lebanon from 22 August through 27 October, 1994. In the first survey 6 species were collected. She resumed collections in mid April 1995 and continued throughout the summer through 13 September 1995, sampling 94 sites in nine transects (50-100km long) along the North and in the mountainous regions of country were sampled. Each transect included localities belonging to one or more of the following climatologic zones: warm Mediterranean Zone, cool Mediterranean zone, and Semi-arid zone. Elevation ranged from 0 to 3,000 meters. *Phlebotomus* species were detected in low densities (111 specimens in 2470 traps). Twelve *Phlebotomus* species have been collected to date, 11 of which are new country records. *Phlebotomus syriacus* was most abundant, followed by *P. jacussieli*, *P. canaaniticus*, *P. brevis*, and *P. simici*. Species of the non-vector genus *Sergentomyia* were far more prevalent, of which specimens of 4 species have been collected. The initial sand fly work was directed more toward species diversity and sand fly distribution than vector incrimination and parasite-sand fly-man interactions. The team should now focus its efforts on sand fly-human-reservoir interactions at known transmission sites.

Objective 3. To identify potential reservoirs associated spatially and temporally with leishmaniasis cases. Dr. Richard Ashford, a well known mammalogist from the Liverpool School of Tropical Medicine, was received on a consultancy visit to help the team identify terrain appropriate for coexistence of both vector sand flies and reservoir animals, and to train the team in trapping and processing animal specimens for *Leishmania* examination. In subsequent field trips to various case sites, Dr. Ashford offered his opinions as to the potential for leishmaniasis transmission based on absence or presence of suitable reservoirs such as rock hyrax or fat sand rats. By the time of this review, further reservoir work had not been reported. The team plans to continue the search for reservoir animals, directing particular emphasis to dogs, the known reservoirs of *L. infantum*.

Summary: The better part of the first two years effort was devoted to achieving the first objective, i.e, preparing for and conducting a national human epidemiology survey. The level of awareness of the disease throughout the country is relatively low but is improving as a result of contacts made with local officials and health care professionals, and as a result of the "propaganda" campaigns carried out in villages and communities where suspected cases were reported. The focus on passive case detection is impractical and the approach should be changed to concentrate on active surveillance in index villages using a standardized skin test. Information gathered thus far indicates that leishmaniasis is sporadic and hypoendemic. Most of the cases detected so far are cutaneous *L. donovani sensu lato*. Only a few cases of *L. major* were reported. As the human epidemiology survey proceeds with greater use of a standardized skin test, a more comprehensive picture of the disease prevalence and distribution will develop. Of concern is the possibility of outbreaks of leishmaniasis due to *L. tropica* that might be introduced by the ½ million migrant workers from Syria. The project should focus some of its attention to determining if conditions are in place to support potential epidemics of *L. tropica* in the future. A reliable, in-house capability for identifying parasites is not yet available and expertise of outside institutions must be depended upon. The team should focus on developing more collaborations within the MERC network to confirm parasite and sand fly identification. A well organized effort has been implemented to achieve the second objective, namely, to identify the potential vectors of leishmaniasis in Lebanon. So far 12 *Phlebotomus* and 4 *Sergentomyia* species have been collected. Potential vectors include *P. papatasi (L. major), P. jacusieli (L. tropica)* and *P. syriacus (L. infantum)*. A poster presentation on the sand flies associated with leishmaniasis case sites in Lebanon was presented at the 1995 annual meeting of the American Society of Tropical Medicine and Hygiene, San Antonio, Texas, and a manuscript is being prepared for publication. During the third year of the study the team should place less emphasis on long-transect trapping through multiple biotopes, and concentrate on sand fly collection at suspected case sites where interaction between parasite, vector, reservoir and host occur. Sticky paper and light trap collections should be supplemented with more vector specific human- and animal-bait collections. Efforts to incriminate reservoir animals have been limited to a consultation visit by Dr. Richard Ashford. During the final year of the study,

special emphasis should be given to dogs as potential reservoirs of *L. infantum*. At the same time, the team should try to collect and examine a wide variety of animals associated with sand fly habitats in infected villages.

Efforts towards establishing regional collaborations: The southern portion of the country is still occupied by Israel and the Hesbollah faction is still powerful, making direct collaboration with Israeli counterparts very problematic. Of the MERC projects, the Lebanese project shows the lowest level of collaboration with the Israelis. The team's reliance on expertise of consultants from outside the MERC network has not contributed to development of ties within the network. However, the PI has exchanged samples and data with, and received monoclonal antibodies from, the Israelis indirectly through NIAID. DNA extracted from parasite cultures has been sent to Dr. Ikram Guizani, Institut Pasteur, Tunis for assistance with typing. Lebanese investigators have participated in semi-annual PI meetings and the PI participated in the workshop in Casablanca that was jointly sponsored by the Moroccans and the Israelis. Expertise is available within the MERC network for confirming parasite identification, vector identification, etc. and the Lebanese team needs to strive to open avenues to tap that expertise.

RECOMMENDATIONS FOR THE FUTURE OF THE PROGRAM:

The review committee felt that the NIAID MERC program has resulted in the development of the foundation of a collaborative network of Israeli and Arab scientists in the Middle East. The combination of workshops, training programs and the semiannual meetings of the principal investigators has served to provide all of the laboratories with an equal technical footing. The standardization of epidemiological methods among the members of the program has also eased the process of sharing and comparing data obtained by the member laboratories. The committee thus feels that the NIAID MERC program has the potential to grow into a regional network with the ability to study diseases important to the Middle East from a regional, as opposed to a national, perspective. Such a network would be valuable not only for the cooperation among scientists in the region it would engender. It would also provide important epidemiological data relevant to the disease problems of the Middle East that would span national borders, much as the diseases themselves do. The committee feels that a major goal of the project in the future will be to build on the accomplishments of the NIAID MERC program to develop such an international network. If this goal could be accomplished, the result would be an important political and scientific success story. The committee has several suggestions for the program that may serve to ensure that the development of such a international network might be realized.

1. The committee felt that the goals of the program would be best met by continuing the multilateral structure of the program as it now exists. The multilateral nature of the program has been one of the most important reasons for the success of the current program, and it should thus be preserved. Within this context, the committee felt that individual bilateral collaborations on specific scientific problems should be encouraged. However, the development of such bilateral collaborative efforts should not be expected to develop more quickly than the local political conditions permit.

2. The committee noted that one of the biggest obstacles to the development of the collaborative relationships has been the difficulty in communications between Israel and her neighboring states. At the start of the program, most of the communications among the groups was routed through Washington. In the past year, with the help of the NIAID, most of the laboratories have gained access to electronic mail. The committee feels that this advance has the potential for eliminating most of the previous communication difficulties. In the future, the

committee suggests that all members of the network be encouraged and perhaps required to have electronic mail capability. The members of the network should be strongly encouraged to use this medium for direct discussion, and the sharing of data and results. The development of a network wide discussion group, similar to the *C. elegans* worm network or the vector biology Bugnet should be considered as one way of facilitating direct communication among the network members.

3. Approximately half of the PI meetings have been held in the US under sponsorship by the NIAID. While this has had some advantages, such as allowing network members to attend international meetings that they otherwise would not have had the opportunity to attend, the committee felt that the members of the network should be asked to carry more of the responsibility of organizing and carrying out these meetings themselves. The committee recommends that the meetings be held at a neutral site close to the Middle East region that is easily accessible to both Arab and Israeli scientists. The responsibility for organizing and sponsoring these meetings could be shared by the members in turn.

4. Many of the activities of the NIAID MERC program have had a parallel structure to date. Thus, many of the groups have concentrated on similar or identical problems in their respective countries. The committee felt that a more efficient approach would be to allow laboratories with similar interests to establish collaborations in which each of the laboratories would concentrate their efforts in those areas where their technical expertise was the strongest. The committee felt that this goal could be approached by allowing certain laboratories within the network to be designated as centers or cores. These laboratories could then serve as a resource for the other members of the network. For example, one of the laboratories might be given responsibility for strain typing parasite isolates, while a second might serve as a core facility for the identification of sandfly vectors. A third laboratory could have responsibility for maintaining a bank of parasite isolates collected from the member laboratories. Such core facilities would ensure that the work was carried out as efficiently as possible, and would serve to further cement the collaborative relationships established within the program.

5. The committee applauds the efforts made to date on the publication of jointly authored abstracts and articles. The committee recommends that the network members continue to make every possible effort to produce jointly authored works. The publication of such works serves not only to document the scientific success of the network, but will also serve as a way to publicize the political success of the program.

APPENDIX 1: STATEMENT OF WORK FOR THE REVIEW

There are three major areas of concern in this mid-term evaluation: 1). efficiency of management, 2) cooperation and collaboration between project countries, and 3) technical progress toward meeting the mid-term objectives on schedule.

EVALUATION CRITERIA. The following components should be considered and addressed in the team's report, as well as additional items based on the professional judgement of the team members. The discussion of each component should be concise, identifying factors affecting implementation in the context of the project purpose and the logical framework; if a framework was included in the project proposal. Recommendations should be confined to significant factors that can be implemented and that will result in increased target beneficiaries. Mid-term evaluations should consider which activities appear to have less potential for success and therefore should receive less resources or none at all.

1 Management: Assess the project management for the following:

- Assess the U.S. coordinator in its back-up role in relation to research coordination, funds flow, report submission, research monitoring, equipment purchasing, workshop/meetings convening, and communicating with all project participants, including AID.

- Are technical reports filed in a complete and timely manner? Do the annual and semi-annual reports contain hard data to verify progress? Do the reports contain a summary section that outlines progress in non-technical terms so that reviewers can easily understand status of the project? Are problems stated and resolutions given?

- Do funds flow to PIs as planned and are financial reports submitted on schedule?

- Does AID respond to requests for clarification, approval, or guidance in a timely fashion?

2. Cooperation: Assess the project implementation for the following:

- How well or to what extent has the project met the goals of the MERC program? In what ways has it been most effective? What aspects have contributed least to meeting the goals?

- How well has NIAID promoted cooperation among the countries of the Middle East and improved the well-being of the people? Which aspects of the program contributed most to the cooperation? Which aspects contributed least?

- How likely are the research projects to solve priority development problems which are recognized by the involved institutions and nations to be of high national priority?

- Has the NIAID project used a collaborative multinational approach to all activities?

- Has the NIAID project been consistent with USAID's priority programs and management concerns in Israel, Tunisia, Morocco, Jordan, and Lebanon?

- What concrete steps have the PIs made towards cooperation with other members of the group? Have there been reciprocal visits, sample or data exchanges, or consultations?

- Have the PIs participated in joint meetings? Have they contributed to the development of a joint work plan and carry out the agenda negotiated?

3. Technical progress: Assess the project implementation for the following:

- Have the research objectives, as originally outlined in the proposal, been met? In the case of changes to the original objectives, have these been appropriate?

- What results and/or conclusions have been drawn? Are they seemingly valid, interesting, and/or valuable?

- Has the number of presentations and publications been adequate, given the duration and nature of the projects?

- How applicable are the results to eventual public health solutions?

B. EVALUATION TEAM COMPOSITION

The evaluation team for the second phase of the evaluation process (site visits in the Middle East) will be comprised of the following participants:

Dr. Brian Bock, AAAS Science, Technology and Diplomacy Fellow, Science, Technology, and Communications Unit, Bureau for Global Programs, Field Support, and Research, US Agency for International Development, Washington, D.C. 20523-1814.

Ms. Avgi Hadjilouca. Director, Laboratory of Infectious Diseases, 15 Acropolis Avenue, Acropolis, Nicosia, 141, Cyprus.

Dr. Philip Lawyer, Chief, Defense Pest Management Information and Analysis Center, AFPMB, Forest Glen Section, WRAMC, Washington, D.C. 20307-5001.

Dr. Franklin Neva, Head, Clinical Parasitology Unit, Laboratory of Parasitic Diseases, Building 4, Room 413, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892.

Dr. Thomas R. Unnasch, School of Public Health, University of Alabama, 720 South 20th Street, Room 203, Birmingham, AL 35294-0008. **Chairman of the Review Committee**

Executive Secretary:

Dr. Kathryn Aultman, Program Officer, Parasitology and International Programs Branch, DMID, Solar Building, Room 3A11, Bethesda, MD 20892.

C: METHODS AND PROCEDURES

Because the program has two distinct goals, the review process was undertaken in two steps. In the first step of the review, the committee[2] met with the principal investigators of the eight projects in San Antonio Texas, on November 16, 1995. The goal of this first stage of the review

[2] Dr. Bock did not attend the first meeting; he participated only in the second stage of the review.

was to evaluate the overall structure of the program, and to assess the success of the program in encouraging regional cooperation among the scientists of the region. The second phase of the evaluation consisted of visits to projects in Israel, Tunisia, Morocco, and Jordan, as well as consultations with the Lebanese PI and her team in Cyprus. Although the emphasis during this phase was primarily on the technical progress made by each project, the site evaluations also provided an additional opportunity to evaluate all phases of the project (management, cooperation, and technical). The second part of the evaluation was carried out in the period March 2 - March 15, 1996.

The team used the following interview, document review, and data collection methods:

1). Review all relevant project and grant papers, progress reports, research reports, and project correspondence in AID-W and the field.

2). Interview PIs and examine activity records, data analyses, and conclusions.

3). Debrief PIs from each country of the findings, as well as brief AID-W, before writing the final evaluation report. The initial drafts prepared by each reviewer were circulated to the other members of the evaluation team for input; the final draft was prepared by the Committee Chairman and circulated again, first to the other reviewers and then to the Principal Investigators for additional comment. These were incorporated by the Executive Secretary.

D. REPORTING REQUIREMENTS

The evaluation report will include an executive summary, project data sheet, table of contents, findings, recommendations, and appropriate appendices (evaluation, scope of work, list of people contacted, actual evaluation schedule, and bibliography of documents reviewed). The body of the report, exclusive of the executive summary and appendices, should not exceed 30 single spaced pages.

The team will formulate their findings, prepare a set of conclusions and from these a set of recommendations for each sub- project. Only serious management, cooperation, and technical concerns should be raised in the recommendations that will help ensure that the objectives of the project can be successfully concluded by the PACD.

E: Funding

Financial support for the evaluation will be supplied by AID.

APPENDIX 2: LIST OF PERSONS CONTACTED

NIAID and Central Project Personnel:

Kathryn S. Aultman, Ph.D., NIAID Program Officer
Peter C. Melby, MD, Leishmaniasis Coordinator
Peter M. Schantz, Ph.D., Hydatid Disease Coordinator

Sub-Project Personnel

Leishmaniasis in Morocco
Nouzha Guessous Idrissi, Ph.D., Principal Investigator
Abdelaziz Hamdani, Ph.D., Entomology
Myriam Riyad, PhD, Parasitology
Soumiya Chiheb, MD, Pediatrics
Boumedienne Berrag, DVM, Veterinary leishmaniasis
Saadia Lasri, immunology

Comprehensive Prophylaxis for Leishmaniasis in Israel
Charles Jaffe, Ph.D., Principal Investigator
Giora Hoida, DVM, Veterinary Studies
Dr. Chaleel Nasara/Tamra*
Dr. Hisham Abu-Rumi/Mayor of Tamra*
Dr. Yosef Malasha/Tamra*[3]

Leishmaniasis in Jordan
Shaden Kamhawi, Ph.D., Principal Investigator
Ali Arbagi, MD, Co-Investigator
Renee Janini, entomology
8 other Team Members

Leishmaniasis in Israel - Parasite-Vector Reciprocal Effects
Prof. Yosef Schlein
Raymond Jacobson, Ph.D.
Prof. Joseph Schlomai

Leishmaniasis in Tunisia
Koussay Dellagi, MD, Principal Investigator
Ikram Guizani, Ph.D.
Hechmi Louzir, MD
Afif Ben Salah, MD

Hydatid Disease in Israel

Prof. Joseph El-On/Ben-Gurion University of the Negev
Dr. Aza Kharebov/Ben-Gurion University of the Negev
Mrs. Ruth Sneir/Ben-Gurion University of the Negev
Dr. Giora Hoida/Ministry of Agriculture, Hadera.
Dr. Michael Furth/Ministry of Agriculture, Hadera.
Dr. Zalman Greenberg/Ministry of Health, Jerusalem.
Dr. Chaleel Nasara/Tamra
Dr. Hisham Abu-Rumi/Mayor of Tamra.
Dr. Yosef Malasha/Tamra

Hydatid Disease in Tunisia

Riadh Ben Ismail, MD, Principal Investigator
Afif Ben Salah, MD, Epidemiology

Leishmaniasis in Lebanon:

Nuha Nuwayri - Salti, M.D.
Efat Abou Fakhr, Ph.D., Entomology
Elias Baydoun, Ph.D, Biochemistry
Khusama Knio, Ph.D, Biochemistry
Zuheir Shbaklo. M.D., Dermatology

APPENDIX 3: EVALUATION SCHEDULE

Saturday 3/2	18:45	Depart Washington Dulles (5 travelers)
Sunday 3/3	15:55 15:05	Arrive Larnaca, Cyprus (5 travelers) Arrive Larnaca, Cyprus (1 traveler)
Monday 3/4	9:00	Briefings re: Lebanese Project
Tuesday 3/ 5	20:30 21:25	Depart Larnaca, Cyprus (7 travelers) Arrive Tel Aviv, Israel; travel by car to Haifa;
Wed 3/6	am: noon	Field visit to Tamra and environs. collect luggage at hotel and travel to Jerusalem; check into
	pm:	briefings by PIs at Hebrew University
Thurs 3/7	am:	briefings by PIs at Hebrew University
	pm:	Field site visit to Jordan Valley, Dead Sea, Masada
Fri 3/8	11:00 2:30	Ground transportation to Amman, Jordan Field trip to the Jordan Valley focus of CL
Sat 3/9	8:30 1:30	visit to laboratory in Irbid Site visits to foci of CL due to *L. tropica*
Sun 3/10	11:25 14:25	Depart Amman Arrive Tunis, Tunisia
Mon 3/11	8:45	Field visits: Medjez el Bab Hospital, Argoub Dispensary, Goubellat Hospital, Khenigat Dhan Dispensary, Bir el Euch Dispensary and field collection sites.
Tues 3/12		Evaluate Tunisian projects: field site visits
Wed. 3/13	14:10 15:55	Depart Tunis; arrive Casablanca, Morocco
Thurs 3/14		Evaluation of Moroccan project
Friday 3/15		Team remains in Casablanca to write up draft report.
Saturday March 16	Depart Casablanca	

APPENDIX 4: LIST OF DOCUMENTS REVIEWED

1. General documents
   Purpose of the Program as a whole
   Description of the Program
   List of Awards
   USAID Guidelines for the MERC Program
   NIAID Proposal to USAID
   Scope of Work from the Agreement between NIAID and USAID
   NIAID MERC Cooperation Activities
   Technical Report #1: September 30, 1993 to March 31, 1994
   Technical Report #2: April 1, 1994 to September 30, 1994
   Technical Report #3: October 1, 1994 to March 31, 1995
   Common Work Plan, Leishmaniasis
   Common Work Plan, Hydatid Disease

2 Projects for individual sub projects

   Proposal to NIAID
   Technical Reports and Work Plans
   Project Progress Summary, where available
   Documentation relating to Cooperation
   List of Program Personnel
   Map of the Country, showing field sites

APPENDIX 5: COOPERATION ACTIVITIES
1994-1996

Workshops

- Molecular Biology of Parasitic Diseases: 8/93, Northampton Massachussetts USA
- Coproantigen Assay for Diagnosis of Canine Echinococcosis: 2/94, itinerant
- Epidemiology Workshop: 4/94, Bethesda
- GIS Workshop: 6/94, Colombo, Sri Lanka
- Biology of Disease Vectors: Heraklion, Crete, 7/94
- Monoclonal Antibodies for Identification of Leishmaniasis: Casablanca, Morocco, 3/95
- Hydatidology, Nicosia, 11/95
- Epidemiology II: Tunis, Tunisia , May 1996 planned
- Leishmania Vaccine Meeting: Amman, Jordan 4/96
- Epidemiology II: Tunis, Tunisia, 9/96

Training/consultation visits:

- Guessous & Riyad to Tunis, 5/94
- Guessous & Hamdani to Amman, 7/94
- Kamhawi to Casablanca, 9/94
- Jaffe to Casablanca, 12/94
- Louzir to San Antonio, 2/95
- Iraqi, Ben Milled to Salford, 2/95
- Blanton & Nieto to Bethesda, 3/95
- Ashford, Guizani to Bethesda, 3/95
- Kamhawi/Ben Ismail to Bethesda, 4/95
- Wilson, Aksoy to San Liurfa, 3/95
- Jaffe to San Liurfa, 5/95
- Ashford to Lebanon & Jordan, 6/95
- Esseghir to Fort Collins, CO, 7/95
- Lasri to Jerusalem, 8/95
- Hamdani & Essari to Tunis, 9/95
- Kasap to Jerusalem, 9/95
- Tawk to Rome, 9/95
- Kamhawi to Venezuela, 10/95
- Kenney to Jordan, 10/95
- Elston to Tunisia, 10/95
- Alissar Chakker to Reims, 3/96
- Louzir, Eisenberger to Bethesda (4/96)
- Schlein, Kamhawi & Abou Fakhr to Casablanca, 6/96
- Guessous to Atlanta Emerging Infectious Diseases (6/96)
- Kamhawi, Janini and Arbagi to Jerusalem, 7/96
- Joseph El On to Cleveland, 8/96
- Rihab Nasr to Tunisia, 10/96
- Handman to Tunisia, 10/96
- Fethi Diwani to Paris, 10/96

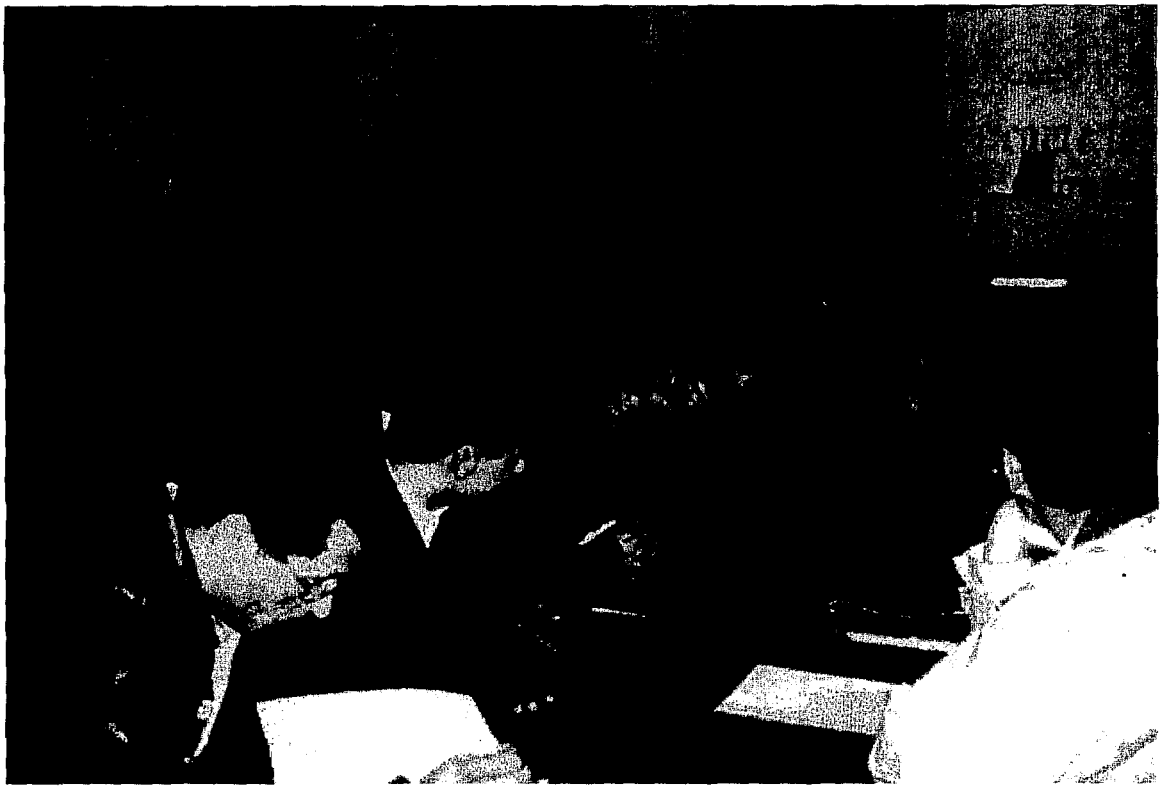
Principal Investigators' Meetings

1. Cairo, Egypt: 9/93
2. Bethesda, Maryland (NIAID ICTDR): 4/94
3. Izmir, Turkey (ICOPA): 10/94
4. Bethesda, MD: 3/95
5. San Antonio, Texas (ASTMH): 11/95
6. Amman, Jordan (WHO/TDR Leishmania Vaccine Meeting): 4/96
7. Baltimore, MD (ASTMH): 12/96
8. Istanbul, Turkey: 5/97

L tropica household in Jordan

The Israeli Hydatid Disease Team

The Tunisian Team



Monoclonal Antibody Workshop in Casablanca, March 1995

The Jordanian Team

MERC Principal Investigators at their April 1996 meeting.

Allenby Crossing

The Casablanca team